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THE SORPTION OF ACIDS BY WOOL¹

BY R. DONOVAN AND P. LAROSE¹

Abstract

The amount of acid sorbed by wool from solutions of sulphuric acid of four different strengths (namely, 0.0505, 0.0339, 0.0182, and 0.0101 molar) and containing sodium sulphate in amounts varying up to 0.16 molar has been determined. It has been found that the presence of the salt has little effect on the quantity of acid sorbed within those limits. The results are analyzed in the light of the theory of Gilbert and Rideal but this theory fails to give a satisfactory explanation of the results obtained. It is possible, however, to explain the results of the authors' experiments on the basis of the recent application of the Donnan equilibrium by Peters and Speakman. The Langmuir adsorption isotherm equation has been applied to data on the absorption of hydrochloric acid and of sulphuric acid by wool. The data appear to fit the Langmuir equation and give, for the maximum combining capacity, values that agree well with those estimated in other ways.

Introduction

The sorption of acids by wool has been studied by a number of investigators and most of the evidence points to a chemical combination between the wool and the acid. Various theories have been advanced to explain the mechanism of acid sorption by wool, but none can be said to be entirely satisfactory from a quantitative point of view, the agreement between theory and experimental results being limited in most cases to a narrow range of the data. However, as this paper was under preparation, Peters and Speakman (12) published a paper in which the Donnan equilibrium is applied with some success to explain the sorption of hydrochloric acid and of sulphuric acid. Elöd (2) and Elöd and Silva (3) have also applied the Donnan equilibrium concept to the sorption of acids by wool, but the agreement between the experimental and the calculated results was only qualitative. The early results of Georgievics (6, 7), Fort and Lloyd (4), Paddon (11), Wilkinson and Tyler (21), and others do not lend themselves to proper quantitative interpretation, since the conditions under which the experiments were carried out were not such as to ensure equilibrium, or the data reported are not sufficiently complete. Harris and coworkers (20) and Steinhardt (18, 19) have used the various dissociation constants of the protein-acid complex to explain the results they obtained with a number of acids. To account for the high sorption of certain weak acids, they postulated direct combination of undissociated acid molecules with the wool.

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Gilbert and Rideal (9) extended to the adsorption of ions on wool an expression given by Fowler and Guggenheim (5, p. 426) for the adsorption of molecules on definite sites of the absorbent. Their theory was successful to some extent when applied to the results of Steinhardt and Harris (17) and to those of Speakman and Stott (16). Gilbert (8) applied the theory to the results obtained by Speakman and Clegg (15) in determining the effect of sodium sulphate concentration on the absorption of a number of acid dyes by wool.

It was evident from a review of the previous work on the subject that further experimental data was required before a general theory could be deduced to explain the acid sorption by wool. With this in mind, the authors decided to study the effect of sodium sulphate on the sorption of sulphuric acid by wool. Although the sorption of the acid itself has been studied, no systematic study seems to have been made of the action of sodium sulphate on the acid sorption. The effect of sodium sulphate on the sorption of the dye Orange II was also studied, but this will form the subject of another paper.

Experimental Procedure

The wool used in our experiments was in the form of a fine worsted yarn that had received no bleaching or other chemical treatment except the usual scouring applied during manufacture. No special purification treatment was given to the wool but it was equilibrated to a pH of 5.7 to 5.8 before the tests by soaking it in a weak acetic acid bath of pH 4.5 until the pH was about 5.1 to 5.2 and then soaking it overnight in distilled water, which brought the pH up to 5.7 to 5.8. The ash in the wool was negligible after this treatment but the wool contained a small percentage ($\sim 1\%$) of fatty and waxy material for which no correction was made. The wool was not dried prior to testing but it was conditioned in a standard atmosphere of 65% R.H. and 21° C., and a control sample was used to determine the moisture content so that the appropriate correction could be made to the samples to convert all results to a dry wool basis.

Samples weighing 2.5 gm. (dry weight) were placed in flasks containing 250 ml. of the solution with known amounts of acid and sodium sulphate. The wool was thoroughly wetted with distilled water and wrung before immersion. Correction for the water adhering to the wool was made by weighing the flask with the wool and solution to determine the exact amount of solution. The flasks were allowed to stand for 64 hr. in a bath maintained at 30° C. Aliquot portions of the solution were then removed for determination of acid and sulphate contents. The difference between the initial and final concentrations was taken as a measure of the sorption. pH determinations were also carried out on the solutions before and after the tests, by means of a model G Beckman pH meter calibrated at a pH of 4.00 with 0.05 *M* potassium acid phthalate. The acid strength was determined by titration with a standard sodium hydroxide solution and phenolphthalein indicator. The sulphate content was determined gravimetrically by precipitation as barium sulphate.

Results

Table I summarizes the results obtained.

TABLE I

AMOUNT OF ACID SORBED BY WOOL FROM SULPHURIC ACID SOLUTIONS
WITH DIFFERENT AMOUNTS OF SODIUM SULPHATE

Na ₂ SO ₄ , moles/liter	pH		Acid sorbed, millimoles/gm.	SO ₄ sorbed, millimoles/gm.
	Before	After		
Original concentration of acid = 0.0505 mole/liter				
0.16	1.55	1.60	0.455	0.356
0.08	1.39	1.45	0.474	0.381
0.04	1.33	1.36	0.396	—
0.02	1.34	1.31	0.448	0.409
0.016	1.24	1.29	0.451	0.430
0.008	1.24	1.27	0.442	0.472
0.004	1.22	1.26	0.402	0.371
0.0008	1.23	1.24	0.419	0.477
0.0000	1.19	1.24	0.452	0.466
Mean			0.438	0.420
Original concentration of acid = 0.0339 mole/liter				
0.16	1.71	1.77	0.409	—
0.08	1.60	1.65	0.427	0.423
0.04	1.48	1.52	0.456	0.422
0.02	1.42	1.46	0.441	0.410
0.016	1.38	1.44	0.443	—
0.008	1.34	1.42	0.424	0.444
0.004	1.36	1.40	0.428	0.386
0.0008	1.34	1.39	0.427	0.451
0.0000	1.32	1.38	0.433	0.435
Mean			0.432	0.424
Original concentration of acid = 0.0182 mole/liter				
0.16	2.06	2.18	0.410	0.331
0.08	1.91	2.01	0.396	—
0.04	1.82	1.91	0.397	0.321
0.02	1.72	1.83	0.427	—
0.016	1.71	1.82	0.398	0.376
0.008	1.69	1.77	0.403	0.420
0.004	1.65	1.75	0.397	0.403
0.0008	1.56	1.67	0.407	0.397
0.00	1.62	1.72	0.389	0.398
Mean			0.403	0.378
Original concentration of acid = 0.0101 mole/liter				
0.04	2.14	2.29	0.378	0.374
0.02	2.00	2.19	0.365	0.362
0.008	1.83	2.03	0.379	0.367
0.004	1.89	2.08	0.364	0.356
0.0008	1.85	2.02	0.365	0.360
0.00	1.83	1.98	0.357	0.357
Mean			0.368	0.363

NOTE: All calculations are based on the weight of dry wool.

Discussion

The results will be examined in the light of the Donnan equilibrium as recently applied by Peters and Speakman (12) and according to the concept introduced by Gilbert and Rideal (9) but before this is done it will be well to refer to the variations noted in the results for any one strength of acid. It will be seen that there is no regularity in the figures obtained for the amount of acid sorbed as the quantity of salt is varied. The differences noted can be readily ascribed to experimental error. With the strengths of acid used, one is working close to the saturation point for wool and the amount of acid sorbed is obtained from the difference of two comparatively large quantities so that any error in the actual strength determination is magnified several times when applied to the amount sorbed. This is emphasized by the observation that the variation in the results becomes less as the acid becomes weaker. The same remarks apply even more to the sulphate determinations, particularly where the salt concentration is high. For example, it has been estimated that the error in the calculated amount of sulphate sorbed from the 0.0505 molar acid containing salt of 0.16 molar concentration is a hundred times the error in the determination of the sulphate in the test solution.

For this reason, the authors do not consider significant the differences between the amount of acid sorbed as determined by change of acidity and the change in sulphate concentration. The acid sorbed is also considered constant for any one strength of acid irrespective of the salt concentration.

The results obtained with the acid solutions without salt are in fair agreement with those reported by Speakman and Stott (16), as Table II shows.

TABLE II

MILLIMOLES OF SULPHURIC ACID SORBED PER GRAM OF DRY WOOL FOR DIFFERENT pH VALUES

pH	Speakman and Stott	Authors' results	
		Acid alone	Acid + salt (Mean)
1.24	0.45	0.45	0.44
1.38	0.44	0.43	0.43
1.72	0.41	0.39	0.40
1.98	0.38	0.36	0.37

Gilbert and Rideal Theory

Gilbert and Rideal (9) in applying their theory to the sorption of sulphuric acid assumed complete dissociation of the acid. Sulphuric acid, however, even in moderately dilute solutions, contains an appreciable proportion of hydrosulphate ions, and for solutions of low pH values this cannot be disregarded, particularly in the presence of sulphates, which increase the sulphate ion concentration of the solution and therefore depress the dissociation of the

hydrosulphate ion. Following the reasoning of Gilbert and Rideal but taking into consideration the hydrosulphate ions, we obtain this expression:

$$\ln \frac{\theta_H}{1 - \theta_H} \cdot \frac{\theta_{SO_4}}{\theta_{HSO_4}} = \ln \frac{[H^+][SO_4^{2-}]}{[HSO_4^-]} - \frac{\Delta\mu_H + \Delta\mu_{SO_4} - \Delta\mu_{HSO_4}}{RT} \quad (1)$$

Both terms on the right hand side are constant, so we may write

$$\frac{\theta_H}{1 - \theta_H} = K \frac{\theta_{HSO_4}}{\theta_{SO_4}}, \quad (2)$$

where K is a constant involving the dissociation constant of the hydrosulphate ion and the affinities of the various ions for wool. However, we have no means of measuring θ_{HSO_4} and θ_{SO_4} separately, the total only being known. Consequently the validity of Equation (1) or Equation (2) cannot be determined readily by experiment. In order to simplify the problem, one could assume that the hydrosulphate ion has a much higher affinity for wool than the sulphate ion and is therefore taken up in preference, or vice versa. If such were the case, the Gilbert and Rideal theory predicts a straight line relation between pH and the logarithm of the anion concentration when θ_H is constant, but our results fail to show such a relation, when a value of 0.88 milliequivalent of acid per gram of wool is taken for the maximum absorption capacity of the wool. It should be pointed out that agreement of the results with the Gilbert and Rideal (9) theory depends to some extent on the choice of this maximum value, but the same lack of agreement was obtained with any reasonable value for the absorption capacity of wool.

As one of the objections to the Gilbert and Rideal theory, Peters and Speakman (12) cite the lack of linear relation between $\log \frac{\theta}{1 - \theta}$ and pH. This objection, based apparently on a value of 0.82 milliequivalent per gram for the combining capacity of wool, is not valid, since Fig. 1 shows that it is possible to obtain a straight line when another value is chosen for the combining capacity. The data of Steinhardt and Harris (17) for hydrochloric acid have been redrawn, with values of 0.90 m.e. (milliequivalent) and 0.82 m.e. for the saturation point. It will be seen that the experimental points lie closely on a straight line when the value of 0.90 is chosen. The objection to the theory of Gilbert and Rideal (9) is rather that it predicts a slope different from that found. A slope of 1 is predicted in this case, whereas the slope of the line in Fig. 1 is 0.80. Use of the activity coefficients would have little effect on this slope.

In an attempt to obtain an independent and convenient way to determine the saturation point for wool, we have applied the Langmuir equation for the adsorption isotherm in the form

$$\frac{c}{x} = \frac{1}{x_m b} + \frac{c}{x_m},$$

where x is the amount of acid sorbed,

x_m is the maximum amount that wool can take up,

c is the concentration of acid in the solution, and

b is a constant dependent on temperature.

Plotting c/x against c gives a straight line with a slope $1/x_m$, from which x_m is determined.

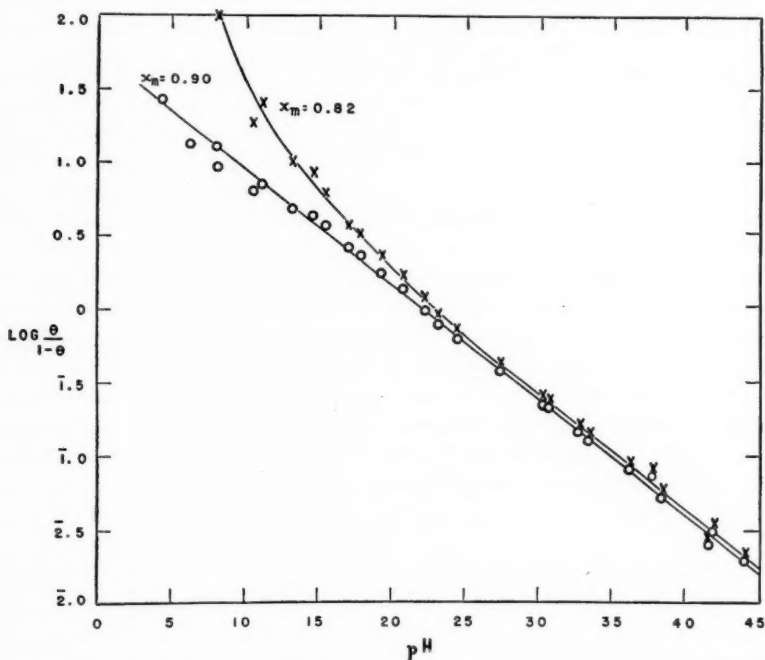


FIG. 1. Results of Steinhardt and Harris (17) for hydrochloric acid sorbed by wool, plotted as a test of the Gilbert and Rideal theory (9). x_m = maximum capacity of wool in milliequivalents per gram.

It should be remembered that the Langmuir equation can be derived from statistical considerations and that it leads to an equation of the same form as that derived by Gilbert and Rideal for acid sorption without salt. The results of Steinhardt and Harris (17) for hydrochloric acid have been plotted according to the Langmuir equation given above in Fig. 2, which gives a slope of 1.17 or $x_m = 0.854$ m.e. per gram of wool. The data of Speakman and Stott (16) for sulphuric acid plotted in Fig. 3 give a slope of 1.14 or $x_m = 0.876$ m.e. per gram. This is the same as the limiting combining capacity found by Peters and Speakman (12) for hydrochloric acid [Case (ii) in their paper]. It may be mentioned also that Lemin and Vickerstaff (10) in their calculations of the affinity of monobasic dyes for wool used a value

of 0.90 milliequivalent per gram for the limiting combining capacity of wool. They found that this represented closely the capacity of wool for the dyes they investigated. There seems to be little doubt then that the figure of

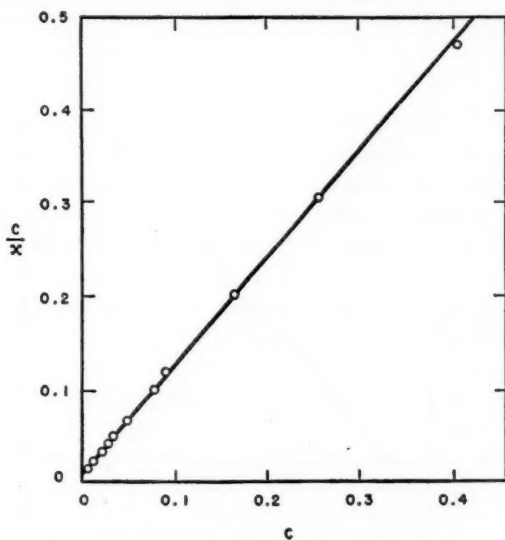


FIG. 2. Results of Steinhardt and Harris (17) for hydrochloric acid sorbed by wool, plotted according to the Langmuir equation.

c = concentration of acid in the solution.

x = amount of acid sorbed.

0.82 m.e. per gram assumed by many investigators to represent the limiting capacity of wool for acids is too low. Speakman and Stott have found a higher apparent combining capacity for sulphuric acid and other acids such as oxalic and phosphoric, but in a recent paper Peters and Speakman have succeeded in explaining this, in the case of sulphuric acid at least, by application of the Donnan equilibrium concept.

Donnan Equilibrium, as Applied by Peters and Speakman

We have followed Peters and Speakman's treatment (12) in applying the Donnan equilibrium to our results, but without neglecting the presence of hydrosulphate ions. We have also attempted to correct for the activity of the sulphate ions.

In order to simplify the calculation of sulphate and hydrosulphate ion concentration we have made the assumption that the activities of the hydrogen ion and of the hydrosulphate ion are the same in the expression

$$\frac{[H^+] \gamma_H [SO_4^{2-}] \gamma_{SO_4}}{[HSO_4^-] \gamma_{HSO_4}} = K = 0.012, \quad (3)$$

where γ is the activity coefficient of the ion designated by the subscript. It is unlikely that the ratio $\gamma_{\text{H}}/\gamma_{\text{HSO}_4}$ is 1 but in view of the low value of γ_{SO_4} , the error introduced by this assumption is probably small. To obtain γ_{SO_4} we

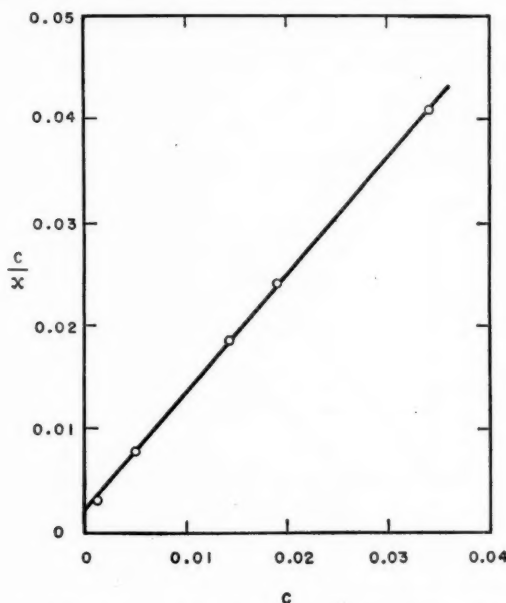


FIG. 3. Results of Speakman and Stott for sulphuric acid sorbed by wool, plotted according to the Langmuir equation.

c = concentration of acid in solution.
 x = amount of acid sorbed.

have followed the practice of Shrawder and Cowperthwaite (14) of taking the value of $\gamma \pm$ for bi-bivalent sulphates and we used the values given by Cowperthwaite and LaMer (1) for zinc sulphate. In order to estimate the SO_4 concentration introduced by the addition of sodium sulphate, it was also necessary to make a correction for the incomplete dissociation of the salt. To do this, we have used the values of Righellato and Davies (13) for the degree of dissociation of the NaHSO_4 ion.

Having obtained the concentrations of HSO_4 and SO_4 ions from Equation (3) we substituted these in Equation (4), given by Peters and Speakman (12),

$$\frac{a}{2v} = \left(\frac{\lambda^2}{\gamma_2} - 1 \right) [\text{SO}_4^-] + \left(\frac{\lambda}{\gamma_1} - 1 \right) [\text{HSO}_4^-], \quad (4)$$

using their notation, where

a = amount of acid combined in the wool,

v = volume of the internal phase,

$$\lambda = \frac{H\gamma_H}{h\gamma_h} \left(\frac{w}{W} \right)^{r_H},$$

$$\gamma_2 = \frac{\gamma_{so_4}}{\gamma_{so_4}} \left(\frac{W}{w} \right)^{r_{so_4}},$$

$$\gamma_1 = \frac{\gamma_{hso_4}}{\gamma_{hso_4}} \left(\frac{W}{w} \right)^{r_{hso_4}},$$

γ is the activity coefficient of the ion designated by the subscript,

r is the ratio of the partial molar volume of the ion to that of water,

w and W are the activities of the water in the internal and external phases respectively, small letters being used to denote quantities in the internal phase and capital letters for the external phase.

The values of v taken are those given by Peters and Speakman for sulphuric acid at corresponding pH. This supposes that the effect of salt addition on the swelling of wool is negligible within the range of concentrations studied. This is perhaps not so, but in the absence of data on the swelling of wool in sulphuric acid solutions containing sodium sulphate, this assumption had to be made. Moreover calculations of the excess of diffusible ions inside the protein over those in the external phase indicate that on the basis of the Procter-Wilson theory of swelling, the effect of salt addition would be expected to be small within the range of concentrations used in our experiments.

γ_1 was assumed to be unity. The ionic strength in the wool phase is different from that in the external phase and one would expect, therefore, the activity coefficient of the ions to be different. It was deemed unnecessary to make the correction, in the case of the monovalent ions, since the concentrations are relatively small and the activity coefficient high, but the concentration of the sulphate ion is comparatively large, being of the order of 1.2 to 1.4 M in the wool phase. Its activity is therefore lower in the wool phase and the ratio

$$\gamma_2 = \frac{\gamma_{so_4}}{\gamma_{so_4}} \left(\frac{W}{w} \right)^{r_{so_4}}$$

must be quite different from one. In calculating γ_2 , it was assumed that the activity of the sulphate ion varies in the same way with ionic strength in the internal phase as it does in the external phase, and the same data were used as in the application of Equation (3) to calculate the concentration of the ions. We have no knowledge of the relative values of w and W , but we have no reasons for believing that the activity of the water is very different in the wool phase from that in the external solution, and $\left(\frac{W}{w} \right)^{r_{so_4}}$ was assumed equal to 1. The values of λ and $vA\theta$ obtained in this way, together with the pH values of the external solution and those calculated for the internal phase, are given in Table III. The results obtained for the solutions with intermediate salt concentrations are omitted as they do not show any behavior different from that of the other salt solutions. When the activity coefficient

TABLE III
VALUES OF pH_i AND $vA\theta$ CORRECTED FOR THE ACTIVITY OF SO_4^{2-} ION

Sodium sulphate conc'n. moles/liter	λ	pH_0	pH_i	$vA\theta$
<i>Final acid concentration, 0.0461 M acid</i>				
0.00	3.64	1.24	1.80	0.852
0.02	3.14	1.31	1.81	0.848
0.04	2.88	1.36	1.82	0.844
0.08	2.48	1.45	1.84	0.844
0.16	2.13	1.60	1.93	0.844
<i>Final acid concentration, 0.0296 M acid</i>				
0.00	3.96	1.38	1.98	0.854
0.02	3.23	1.46	1.97	0.852
0.04	2.82	1.52	1.97	0.848
0.08	2.40	1.65	2.03	0.846
0.16	2.08	1.77	2.09	0.846
<i>Final acid concentration, 0.0142 M acid</i>				
0.00	4.53	1.71	2.37	0.804
0.02	3.28	1.83	2.35	0.800
0.04	2.80	1.92	2.37	0.798
0.08	2.40	2.01	2.39	0.798
0.16	2.05	2.18	2.49	0.798
<i>Final acid concentration, 0.00642 M acid</i>				
0.00	5.44	1.98	2.72	0.732
0.02	3.31	2.19	2.71	0.730
0.04	2.71	2.29	2.72	0.728
0.00*	5.36	1.98	2.71	0.765

* Peters and Speakman data.

pH_0 = pH of external solution,

$vA\theta$ = true amount of combined acid,

pH_i = pH of internal phase,

v = volume of internal phase,

A = maximum combining capacity of wool expressed as a concentration,

θ = fraction of such capacity that has been satisfied through the combination of RCOO^- and H^+ ions.

of the sulphate ion is not taken into consideration, the values of pH_i are higher and the amount of acid combined lower.

Table III indicates that the saturation value for acid absorption is 0.85 to 0.86 m.e. per gram, which agrees very well with the figures given by Peters and Speakman (12) although their saturation value was obtained at a lower pH .

In order to determine the effect of the sulphate ion activity on the results given by Peters and Speakman, we have used their data for an external pH of 1.98, since this is the only point that did not require interpolation to compare with our data. The values obtained for λ , pH_i , and $vA\theta$ are given in the last line of Table III. The value of combined acid obtained differs little from the value of 0.757 m.e. per gm. given by the above authors but differs appreciably from our value of 0.732 for an acid of 0.00642 *M*. However Peters and Speakman give the concentration of their acid as 0.00705 *M*. The difference corresponds to 0.04 pH difference, and, from our results, this might be expected to raise the acid combined from 0.732 to 0.742 m.e. per gm.

The agreement between the value of 0.757 given by Peters and Speakman and our calculated value of 0.765 is perhaps due to the fact that the error arising from the neglect of the sulphate ion activity coefficient in calculating the concentrations of the ions in the solution cancels the error that results from neglecting the activity in calculating the concentration in the internal phase.

It would appear from these results that although the explanation given by Peters and Speakman explains satisfactorily the experimental observations, a more rigorous test of the theory is desirable by means of experiments in which the concentration of the ions, the amount of acid combined, and the degree of swelling of the wool are determined more accurately. More should also be known of the activities of the ions, particularly in the wool phase. Experiments are being planned to fill this need.

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THE REDUCTION OF CERTAIN CHLORONITROBENZENES BY DEXTROSE AND BY SODIUM ARSENITE¹

By ROGER GAUDRY AND KARL F. KEIRSTEAD

Abstract

The reduction of seven chloronitrobenzenes to azoxy compounds by alkaline solutions of two different reducing agents, dextrose and sodium arsenite, has been studied. These two reducing agents were found to produce a better yield and a purer product than many other reducing agents. Two azoxy compounds have been prepared for the first time: 2,2'-dimethyl-3,3'-dichloroazoxybenzene and 2,2'-dimethoxy-5,5'-dichloroazoxybenzene.

Introduction

The reduction of nitrobenzene to azoxybenzene was first carried out in 1841 by Zinin (see (1)) who used alcoholic potassium hydroxide. Since that time nitrobenzene compounds have been reduced to azoxy and related compounds most frequently by the use of either alcoholic potassium hydroxide or by sodium alcoholate in alcohol solution. The applicability of these reducing agents is however limited by such factors as poor yields, impure products, or violent reactions.

The present investigation was undertaken primarily to study the general applicability of two reducing agents, sodium arsenite and dextrose, to the preparation of azoxy compounds. The use of dextrose in alkaline solution for the reduction of nitrobenzene compounds has been reported for the preparation of azoxybenzene (13) and 2,2'-dichloroazoxybenzene (9). Sodium arsenite as a reducing agent for the preparation of azoxybenzene was first used by Loesner (11). The successful application of sodium arsenite for the preparation of azoxy derivatives (2, 3) suggests that this reducing agent may have many advantages.

We found that dextrose gave better yields than sodium arsenite in the following cases: 2-chloronitrobenzene, 2,5-dichloronitrobenzene, 2-methyl-3-chloronitrobenzene, and 2-methoxy-5-chloronitrobenzene—all of which have a substituent in the ortho position. The experimental conditions and results are shown in Tables I, II, and III.

By using dextrose we obtained a 27% yield of 2,2'-dichloroazoxybenzene as compared with a yield of 11% obtained by the use of sodium arsenite. The low yield in the latter reduction may partly be explained by the results of Dains and Kenyon (6), who showed that in the reduction of 2-chloronitrobenzene by sodium methylate 7.25% of the chlorine is removed; 12.72% of the chlorine is removed in the reduction of 4-chloronitrobenzene, while no removal is observed in the reduction of 3-chloronitrobenzene under similar conditions.

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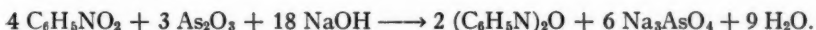
Contribution from the Department of Biochemistry, Faculty of Medicine, Laval University, Quebec. This paper constitutes part of a thesis submitted by Karl F. Keirstead to the Graduate School, Laval University, in partial fulfillment of the requirements for the degree of Doctor of Science.

We found that at the end of the reaction the solution containing the dextrose-chloronitrobenzene mixture gave a white precipitate on the addition of silver nitrate. The low yields produced then by both sodium arsenite and dextrose on 2-chloronitrobenzene and 4-chloronitrobenzene are due, in part, at least, to the tendency of the chlorine in the ortho and para positions to a nitro group to be removed in the presence of a reducing agent in strong basic solution.

However, the loss of chlorine is not sufficient to explain entirely the low yields in the reactions just mentioned since the percentage of recovered chloronitrobenzene is greatest with ortho and para chloronitrobenzene; this indicated that some factor inhibits the reduction of the nitro group.

The temperature conditions for the reduction by dextrose were those found by Opolonick (13) to give the best yields of azoxybenzene, viz., a fairly low temperature during the addition of the dextrose, followed by a higher temperature for the remainder of the reaction. The yield of the crude azoxy compound is given in those cases where the product is slightly dark but easily purified. It may be noted that the use of dextrose in alkaline solution can be expected to be a good method for the preparation of the amine when the solution is more dilute. Such an example is found in Expt. 9, Table I.

The reaction for the reduction of a nitrobenzene compound by sodium arsenite is:



In practice an excess of sodium arsenite, as well as a still greater excess of sodium hydroxide, is used to increase the yield. In some experiments methyl alcohol was added to the chloronitrobenzene - sodium arsenite mixture in an attempt to improve conditions by increasing the solubility of the chloronitrobenzene compound. In two experiments, by the reduction of 2-chloronitrobenzene and 2,5-dichloronitrobenzene, an entirely new product appeared, which is a hydroxyazo compound. The analysis of the product produced by the reduction of 2-chloronitrobenzene indicates that it is an isomer of 2,2'-dichloroazoxybenzene. It was not studied further except to note that its physical constants do not correspond to those of 4-hydroxy-2,2'-dichloroazobenzene.

To gain experience we carried out reduction of chloronitrobenzene compounds by these reducing agents: magnesium in methyl alcohol (15), magnesium in methyl alcohol and saturated ammonium chloride solution (14), zinc dust in alcohol and saturated ammonium chloride solution (5), sodium methoxide in absolute alcohol, sodium ethoxide in absolute alcohol, and potassium hydroxide in absolute alcohol. We found that reduction by dextrose or by sodium arsenite had the advantages of producing better yields and a product more easily purified.

Experimental

Reduction of a Chloronitrobenzene Compound. Tables I, II, and III

In each case the experimental conditions are found in the tables. At the end of the reaction time any alcohol present was removed by distillation. The remaining solution was then steam distilled from basic solution until no further solids appeared in the steam distillate. This required 3 to 24 hr., the actual time depending on the amount of the amine and unchanged chloronitrobenzene present in the mixture and on the vapor pressure of these products.

The residue remaining from the steam distillation in the dextrose reduction was digested with large volumes of hot water to remove water soluble impurities. When the azoxy compound was very black or dark red, as in Expts. 3, 4, and 9, Table II, it was first washed with cold alcohol, in which the azoxy compound is only very slightly soluble. This treatment removes most of the tars. In other cases, as in Expt. 7, Table II, the azoxy compound was dissolved in benzene and warmed for a short time with charcoal to remove tars. Any remaining red substance, as in Expts. 3, 4, and 9, Table II, was removed by digesting in portions with a large volume (2 to 10 liters) of a dilute solution of sodium hydroxide or sodium carbonate. The filtrate containing the hydroxyazo compound was made acid. The red precipitate formed was removed by filtration, and, when dry, it was crystallized from

TABLE I
REDUCTION OF SOME NITROBENZENE COMPOUNDS* BY DEXTROSE

Expt. No.	Substituent	% recovery	Reagents			Reaction conditions: time, hr. temp., °C.	Azoxy, % yield	Other products, % yield
			Dextrose, moles	NaOH, moles	H ₂ O, ml.			
1	2-Chloro	42	0.25	1.5	200	1/70 + 2/90	27c, 8r	—
2	3-Chloro	0	0.23	1.5	200	1/60 + 2/110	51 c	—
3	4-Chloro	45	0.23	1.5	200	3/109	22.6 c, 12 r	—
4	2,5-Dichloro	0	0.23	1.5	300	3/90	25 c	—
5	3,4-Dichloro	20	0.23	1.5	300	1/70 + 2/85	20 c	—
6	Same	17	0.5	1.5	800	1/60 + 2/110	17 c	Amine (0.5 gm.)
7	2-Methyl-3-chloro	98	0.23	1.5	200	1/70 + 2/109	2 c	—
8	Same	26	0.6	1.5	300	1/70 + 2/109	25.4 c	Amine, 4.5
9	2-Methoxy-5-chloro	1.5	0.6	1.5	300	1/70 + 2/109	49 c	Amine, 21

* The amount of nitrobenzene compound used in each case was 0.33 mole.

Note: (a) Mechanical stirring in each case.

(b) Dextrose added during first hour, in small portions.

(c) % yield = % of the theory.

(d) c = Crude.

r = Refined.

TABLE II

REDUCTION OF SOME NITROBENZENE COMPOUNDS* BY SODIUM ARSENITE

Expt. No.	Substituent	% re-covery	Reagents				Reaction conditions: time, hr. temp., °C.	Azoxy, % yield	Other products, % yield
			As ₂ O ₃ , moles	NaOH, moles	H ₂ O, ml.	CH ₃ OH, ml.			
1	2-Chloro	45	0.275	1.5	200	—	8/75	7 r	—
2	Same	57	0.275	1.7	200	—	8/90	11 c	—
3	Same	9	0.275	1.7	300	200	8/90	10 c	Hydroxyazo, 5
4	Same	14	0.250	1.5	125	250	6/48	7 c	Amine, 21, Hydroxyazo, 9
5	3-Chloro	9	0.275	1.7	180	—	8/90	82 c	—
6	4-Chloro	9	0.275	1.7	200	—	8/109	48 c	—
7	2,5-Dichloro	0	0.275	1.7	300	—	8/105	9 c	Amine, 14
8	Same	18	0.275	1.6	200	—	8/90	21 c	—
9	Same	11	0.284	1.62	60	60	6/74	26 c	Hydroxyazo (1.9 gm.)
10	3,4-Dichloro	25	0.275	1.7	200	—	10/85	35 c, 24 r	—
11	2-Methyl-3-chloro	0	0.275	1.7	200	—	8/109	0	Amine, 12
12	Same	61	0.275	1.7	200	100	5/79	14 r	Amine, 5
13	Same	56	0.275	1.7	300	100	8/79	6 c	—
14	2-Methoxy-5-chloro	13	0.275	1.7	200	200	8/79	36 r	Amine, 10

* The amount of the nitrobenzene compound used in each case was 0.33 mole.

Note: (a) Mechanical stirring in each case.

(b) % yield = % of theory.

(c) Stoichiometric proportions: Nitrobenzene compound, 0.33 mole; arsenic trioxide, 0.25 mole; sodium hydroxide, 1.5 moles.

(d) c = Crude.

r = Refined.

benzene. The azoxy compound after treatment with the cold alcohol and the dilute basic solution was fairly pure and was recrystallized several times from a mixture of alcohol and benzene.

The steam distillate consists of unchanged chloronitrobenzene and any amines formed. The solids in the steam distillate were digested in acid solution and filtered. The residue was unchanged chloronitrobenzene. In some cases the residue from the acid digestion was an oily liquid from which the chloronitrobenzene crystallized only after standing in the ice box for several days. The amine was precipitated by making the acid solution basic. When the amine was a liquid or was present in small amounts, as in Expt. 6, Table I, the basic solution was extracted with ether. The ether extracts were evaporated and the residue converted into the acetyl derivative for the determination of the melting point or for analysis in doubtful cases.

The solution remaining from the steam distillation was made neutral and extracted with ether. In no case was more than a trace of a phenol found.

TABLE III
AZOXYBENZENES PRODUCED BY REDUCTION OF SUBSTITUTED NITROBENZENES

Compound	Formula	M.p., ° C. (un-corrected)	Color	Analysis	
				Calc.	Found
2,2'-Dichloroazoxybenzene	$C_{12}H_8ON_2Cl_2$	56 ^a	Pale yellow	N, 10.48 C, 53.95 H, 3.01 Cl, 26.54	10.43 53.93 3.08 26.57
3,3'-Dichloroazoxybenzene	$C_{12}H_8ON_2Cl_2$	97 ^b	Dark yellow	Cl, 26.54	25.78
4,4'-Dichloroazoxybenzene	$C_{12}H_8ON_2Cl_2$	155 ^c	Light yellow	Cl, 26.54	25.53
2,2',5,5'-Tetrachloroazoxybenzene	$C_{12}H_4ON_2Cl_4$	147 ^d	Faint yellow	N, 8.63	7.35
3,3',4,4'-Tetrachloroazoxybenzene	$C_{12}H_4ON_2Cl_4$	139 ^e	Lemon yellow	Cl, 42.20	41.38
2,2'-Dimethyl-3,3'-dichloroazoxybenzene	$C_{14}H_{12}ON_2Cl_2$	122-123 ^f	Faint yellow	Cl, 24.02	23.41
2,2'-Dimethoxy-5,5'-dichloroazoxybenzene	$C_{14}H_{12}O_2N_2Cl_2$	119 ^g	Brownish yellow	Cl, 21.67	22.99

^a Ref. (4) 56° C.

^b Ref. (10) 97° C.

^c Ref. (8) 155° to 156° C.

^d Ref. (7) 147° C.

^e Ref. (6) 137° to 138° C.

^f New compound.

^g New compound.

A detailed description of one case each of reduction by dextrose and by sodium arsenite follows.

Reduction of 3,4-Dichloronitrobenzene by Dextrose

A mixture of sodium hydroxide (60 gm., 1.5 moles), water (750 ml.), and 3,4-dichloronitrobenzene (63 gm., 0.33 mole) was heated with efficient stirring in a 1-liter, 3-necked flask at 60° to 70° C. for one hour. During this time a syrup of dextrose (45 gm., 0.23 mole) in water (50 ml.) was added in small portions. If necessary the flask was cooled to keep the temperature below 70° C. At the end of the first hour the temperature was allowed to rise to 110° C., at which point it was maintained for the last two hours.

The reaction mixture was then steam distilled until the amount of solid coming over in the steam distillate was negligible. The distillate was made acid, warmed, and filtered. The residue was unchanged 3,4-dichloronitrobenzene. Yield, 11 gm. (17% of the original); m.p., 41° C.

The filtrate was made basic with sodium hydroxide. A light precipitate of 3,4-dichloroaniline was obtained. The mixture was filtered. The clear solution, which smelled of 3,4-dichloroaniline, was extracted with ether. When the ether had evaporated, the residue was added to the 3,4-dichloroaniline precipitated from the solution. Combined yield was 0.5 gm.; m.p.

71° C. Found (Kjeldahl): N, 8.55%. Calc. for $C_6H_5NCl_2$: N, 8.64%. Found (Na_2O_2 fusion): Cl, 43.41%. Calc. for $C_6H_5NCl_2$: Cl, 43.77%.

Acetyl derivative: m.p., 122.5° C. Found (Kjeldahl): 6.56%. Calc. for $C_8H_7ONCl_2$: N, 6.56%. Found (lime fusion): Cl, 34.14%. Calc. for $C_8H_7ONCl_2$: Cl, 34.76%.

The original solution remaining from the steam distillation contained a fairly heavy deposit of brown granular 3,3',4,4'-tetrachloroazoxybenzene, which was removed from the solution by filtration. The azoxy compound was treated with hot water until all water soluble substances were removed. The compound was dried and weighed; yield, 19 gm. (17% of the theoretical). An almost quantitative yield was obtained when the compound was crystallized from a mixture of alcohol and benzene; m.p., 139° C. (For analysis see Table III.)

Reduction of 2-Methyl-3-chloronitrobenzene by Sodium Arsenite

To a thick paste of arsenic trioxide (54.4 gm., 0.275 mole) and water was slowly added with stirring a solution of sodium hydroxide (70 gm., 1.7 moles) and water (200 ml.). The mixture was poured into a 1 liter, three-necked flask. To this mixture was added a warm solution of 2-methyl-3-chloronitrobenzene (57.2 gm., 0.33 mole) dissolved in methyl alcohol (100 ml.). The whole mixture was stirred for five hours at 79° C. A heavy yellow oil slowly formed. At the end of the time the reaction mixture was treated as for reduction by dextrose.

After the steam distillation, a yellow granular deposit of almost pure 2,2'-dimethyl-3,3'-dichloroazoxybenzene was obtained. Yield, 13.5 gm. (14% of the theoretical). The analysis is found in Table III. The acetyl derivative of 2-methyl-3-chloroaniline melted at 157° C. (12). Yield, 3 gm. Found (lime fusion): Cl, 18.55%. Calc. for $C_9H_{10}NOCl$: Cl, 19.31%. Recovered: 34.5 gm. (61% of the original) unchanged 2-methyl-3-chloronitrobenzene; m.p. 37° C.

Acknowledgment

Generous samples of 2-chloronitrobenzene and 2,5-dichloronitrobenzene were received from Monsanto, Ltd. This gift is gratefully acknowledged. One of us (K.F.K.) wishes to express his indebtedness to the late Dr. H. E. Bigelow, then Carnegie Professor of Chemistry, Mt. Allison University, for his valued advice regarding the reduction of 2-chloronitrobenzene and 2,5-dichloronitrobenzene by sodium arsenite. We are indebted to an unpublished thesis by H. J. Hockin, Mt. Allison University, for certain suggestions regarding the reduction of 2-chloronitrobenzene by sodium arsenite.

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THE IDENTIFICATION OF AZOXY COMPOUNDS¹

BY ROGER GAUDRY AND KARL F. KEIRSTEAD

Abstract

An azoxy compound can be treated with concentrated sulphuric acid to produce a hydroxyazo product consisting chiefly of the para isomer. This reaction, the Wallach rearrangement, is the basis for a suggested generic test for an azoxy compound. For the identification of a given azoxy compound, two derivatives are suggested: the hydrazo compound produced by the reduction of the azoxy compound by zinc dust, and the azo compound produced by the oxidation of the hydrazo compound. These compounds are described for the first time: 2,2'-dichloro-4-hydroxyazobenzene, 2,5',2,5'-tetrachloro-4-hydroxyazobenzene, 2,2'-dimethoxy-5,5'-dichloroazobenzene, 3,3',4,4'-tetrachlorohydrazobenzene, 2,2'-dimethyl-3,3'-dichlorohydrazobenzene, and 2,2'-dimethoxy-5,5'-dichlorohydrazobenzene.

Introduction

Most types of compounds have some well developed and convenient method for their identification. In the case of azoxy compounds, the general suggestions found in the literature are too vague for convenient use. As a typical example we find in Shriner and Fuson's text (16, p. 172) the following suggested procedures:

- (1) Reduction of the azoxy compound by tin to a known amine,
- (2) Reduction of the azoxy compound to a known azo compound by suitable reagents.

The first procedure is, of course, not a specific test for an azoxy compound since nitro, azo, and azoxy compounds can all be reduced to an amine by tin. The latter procedure is not given in detail and the table of azo compounds given in Shriner and Fuson's text is not extensive enough to be of much use. It would be desirable then to have (a) a generic test for azoxy compounds, and (b) a table of melting points of derivatives that can be prepared conveniently from azoxy compounds.

The reaction, known as the Wallach rearrangement (18) appears to be well suited as a generic test for azoxy compounds. The reaction consists of the conversion of an azoxy compound into a hydroxyazo compound by the action of concentrated sulphuric acid.

Bamberger (1) showed that a small amount of ortho hydroxyazobenzene was formed as well as the para hydroxyazo compound. Lachman (10), who made the most careful study of the rearrangement of azoxybenzene, reported that 1.5 gm. of 2-hydroxyazobenzene was obtained from the rearrangement of 75 gm. of azoxybenzene. If the para position is occupied, as in 4,4'-dichloroazoxybenzene, the oxygen is removed entirely. In the latter reaction, the product is 4,4'-dichloroazobenzene (7).

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Contribution from the Department of Biochemistry, Faculty of Medicine, Laval University, Quebec, Que.

This rearrangement has been used by some investigators (12, 13, 15, 18) for the preparation of hydroxyazo compounds.

We have studied the Wallach rearrangement and the chief product of a number of chloroazoxybenzene compounds whose preparations are described in a previous paper (6). The condition for each rearrangement and the chief product are shown in Table I.

In each experiment the hydroxyazo compound was carefully purified. Judging from the rearrangement of azoxybenzene (10) and of 4-nitroazoxybenzene (12), we would expect the chief product to be the para hydroxy compound. We attempted to establish the constitution and to judge the purity of the product formed by the rearrangement of 2,2'-dichloroazoxybenzene by preparing, according to the method of Schoutissen (14), 2,2'-dichloro-4-hydroxyazobenzene by coupling diazotized 2-chloroaniline and 3-chlorophenol. A comparison of the physical properties of the compound produced by rearrangement and the compound prepared by synthesis shows fairly good agreement. It is quite certain that the former compound is fairly pure 2,2'-dichloro-4-hydroxyazobenzene.

From all available data, the following procedure is a generic test for azoxy compounds: the compound when treated with a large volume of concentrated sulphuric acid (*a*) becomes bright red and is soluble in the acid, (*b*) is precipitated when the mixture is poured into water, and (*c*) is, after that, fairly soluble in dilute basic solution.

An azoxy compound may be reduced conveniently and quantitatively to a hydrazo compound which, in turn, may easily be oxidized to the azo compound. We suggest that the hydrazo and azo compounds so formed be used as derivatives for the identification of an azoxy compound. A list of azo and hydrazo derivatives of the azoxy compounds described in a previous paper (6) is found in Table II.

Experimental

Wallach Rearrangement

The azoxy compound (10 gm.) was rapidly mixed with concentrated sulphuric acid (100 gm.). The mixture rapidly became red. However, this color does not necessarily indicate that the reaction has gone very far. Where necessary the mixture was warmed on a water bath. After heating for the minimum time as indicated by several trials, the mixture was poured into an excess of water. The precipitated solid was digested with a large volume of dilute sodium carbonate solution (2 to 10 liters). The dark residue of polymerized products remaining after this treatment was discarded. The free hydroxyazo compound was precipitated by making the combined filtrates neutral by addition of hydrochloric acid. The precipitated hydroxyazo compound was colloidal; this made ordinary suction filtration almost impossible. After the mixture was allowed to stand for some time most of the liquid was decanted. The remainder of the liquid was removed by allowing the mixture to stand overnight in a large Büchner funnel. After the residue had dried, the compound was purified by repeated precipitation from a mixture of alcohol

TABLE I
 CHIEF COMPOUND PRODUCED BY WALLACH REARRANGEMENT

Substituents	Conditions	M.p., °C., uncor- rected	Color	Formula	Analysis		Derivative
					Calc.	Found	
2,2'-Dichloro- (4-hydroxy)	Cold conc. H ₂ SO ₄	^b 174 (185)	Light brown	C ₁₂ H ₈ ON ₂ Cl ₂	Cl, 26.54	25.38	Benzoyl, m.p. 132°C.
2,2'-Dichloro- 4-hydroxy	"	178 (183)	Light brown	C ₁₂ H ₈ ON ₂ Cl ₂	—	—	^a Benzoyl, m.p. 152°C.
3,3'-Dichloro	30 min. in conc. H ₂ SO ₄ , 150°C.	125	Brown	C ₁₂ H ₈ ON ₂ Cl ₂	—	—	—
^a 2,2',5,5'-Tetra- chloro (4-hyd- roxy)	20 min. in conc. H ₂ SO ₄ , 100°C.	191	Red	C ₁₂ H ₆ ON ₂ Cl ₄	Cl, 42.20	41.41	Benzoyl, m.p. 210°C.
3,3',4,4'-Tetra- chloro	20 min. in conc. H ₂ SO ₄ , 100°C.	202	Yellow	C ₁₂ H ₆ ON ₂ Cl ₄	—	—	—
2,2'-Dimethyl- 3,3'-dichloro	20 min. in conc. H ₂ SO ₄ , 100°C.	163 (157)	Brown	C ₁₄ H ₁₀ ON ₂ Cl ₂	Cl, 24.02	25.81	—

* By synthesis.

^a Found: Cl, 19.62%. Calc. for C₁₂H₁₂N₂Cl₂O₂: Cl, 19.10%.

^b Melting point varies with rate of heating.

^c Structure determined by identification of products produced by reduction of the compound by stannous chloride.

and benzene until a constant melting point, under the same conditions, was reached. The melting point depends somewhat on the rate of heating.

Hydrazo Derivative of the Azoxy Compound

The azoxy compound (10 gm.) was dissolved in the minimum amount of alcohol to which 40% solution of sodium hydroxide (35 ml.) was added. The mixture was refluxed on a steam bath with excess zinc dust. The mixture became red in a short time. The heating was continued for several hours until the solution became colorless or almost so. The solution was removed from the zinc by decantation. After the solution had cooled, the hydrazo compound was precipitated by the addition of a small amount of water. If the precipitate had more than a trace of color, it was dissolved in alcohol containing a few drops of acetic acid and warmed with a little zinc dust for a few minutes, after which the colorless hydrazo compound was precipitated as before, and dried in a vacuum desiccator.

Azo Derivative of the Azoxy Compound

The hydrazo compound was dissolved in alcohol and oxidized by boiling in the open air or by bubbling air through the warm solution. The azo compound, being less soluble than the hydrazo compound, readily settles out of the alcohol.

TABLE II
AZO AND HYDRAZO DERIVATIVES OF CERTAIN AZOXY COMPOUNDS

Substituents	Azobenzene		Hydrazobenzene	
	Formula	M.p., °C.	Formula	M.p., °C.
2,2'-Dichloro	C ₁₂ H ₈ N ₂ Cl ₂	136 ^a	C ₁₂ H ₁₀ N ₂ Cl ₂	87 - 88 ^b
3,3'-Dichloro	C ₁₂ H ₈ N ₂ Cl ₂	99 ^b	C ₁₂ H ₁₀ N ₂ Cl ₂	94 ⁱ
4,4'-Dichloro	C ₁₂ H ₈ N ₂ Cl ₂	185 - 186 ^c	C ₁₂ H ₁₀ N ₂ Cl ₂	122 ^j
2,2',5,5'-Tetrachloro	C ₁₂ H ₆ N ₂ Cl ₄	188 - 189 ^d	C ₁₂ H ₈ N ₂ Cl ₄	124 ^k
3,3',4,4'-Tetrachloro	C ₁₂ H ₆ N ₂ Cl ₄	157 ^e	C ₁₂ H ₈ N ₂ Cl ₄	136 - 137 ^l
2,2'-Dimethyl-3,3'-dichloro	C ₁₄ H ₁₂ N ₂ Cl ₂	154 ^f	C ₁₄ H ₁₄ N ₂ Cl ₂	138 - 139 ^m
2,2'-Dimethoxy-5,5'-dichloro	C ₁₄ H ₁₂ N ₂ Cl ₂ O ₂	201 ^g	C ₁₄ H ₁₄ N ₂ Cl ₂ O ₂	118 ⁿ

^a Ref. (2), 136° C. Calc.: Cl, 28.10%. Found: Cl, 28.01%.

^b Ref. (11), 101° C.

^c Ref. (19), 185° to 186° C.

^d Ref. (4), 189° C.

^e Ref. (9), 195.5° C. (impure).

^f Ref. (3), 154° C. Calc.: Cl, 25.40%. Found: Cl, 26.05%.

^g New compound. Calc.: Cl, 22.79%. Found: Cl, 21.55%.

^h Ref. (17), 87° to 88° C.

ⁱ Ref. (11), 94° C.

^j Ref. (8), 122° C.

^k Ref. (5), 123.5° C. Ref. (4), 117° C.

^l New compound. Calc.: Cl, 44.03%. Found: Cl, 43.60%.

^m New compound. Calc.: Cl, 25.22%. Found: Cl, 23.98%.

ⁿ New compound. Calc.: Cl, 22.69%. Found: Cl, 21.65%.

A second crystallization from alcohol gave a pure azo compound with a sharp melting point.

2,2'-Dichloro-4-hydroxyazobenzene

2-Chloroaniline (3 gm.) was dissolved in concentrated sulphuric acid (15 ml.). To this solution, cooled to 0° C., was added nitrosylsulphuric acid, which was prepared by mixing concentrated sulphuric acid (15 ml.) with sodium nitrite (3.5 gm.) with cooling and mechanical stirring to keep the temperature of the mixture between 0° and 10° C. The temperature was kept below 10° C. while concentrated phosphoric acid (50 ml.) was added with vigorous mechanical stirring. After 30 min., powdered urea (2 gm.) was added to decompose excess nitrous acid, and the solution was poured on cracked ice. The faintly yellow solution was poured slowly, with cooling, into a mixture containing 3-chlorophenol (3 gm.) and sodium hydroxide (214 gm.).

After standing for a few minutes, the soluble portion was removed by boiling with water (3 × 400 ml.). A dark brown residue, with high melting point, remains. Dilute hydrochloric acid was added to the solution containing the water soluble portion. The precipitate was purified in the same way as the similar compounds prepared by the Wallach rearrangement.

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REDUCTION OF STEROIDS USING LITHIUM ALUMINUM HYDRIDE¹

BY G. PAFINEAU-COUTURE, E. M. RICHARDSON², AND GORDON A. GRANT

Abstract

Lithium aluminum hydride has been used successfully in the partial reduction of phthalic anhydride to phthalide, in the selective reduction of the carbonyl group of dehydroisoandrosterone-3-acetate, and in the reduction of oestrone acetate to α -oestradiol without simultaneous production of the β -isomer. A new reduction product of Δ^5 -3(β)hydroxyetiobilenic acid, $\Delta^{5,14}$ -2,13-dimethyl-7(β)hydroxy-2-hydroxymethyl-1-hydroxyethyl dodecahydrophenanthrene, is also described.

Introduction

It is often desirable to reduce the carbonyl group of esterified ketosteroids without hydrolyzing or reducing the ester group. In general, usual reagents such as zinc-hydrochloric acid or aluminum isopropylate give relatively low yields. These reactions are slow and are carried out in the presence of an excess of the reagent, so that several simultaneous reductions can take place. The properties of the recently discovered lithium aluminum hydride (LiAlH_4) suggested that it might be a more selective reducing agent.

Lithium aluminum hydride was first synthesized by Finholt, Bond, and Schlesinger (2). Its use as a reducing agent in organic chemistry was first reported by Brown, Finholt, Nystrom, and Schlesinger at the New York meeting of the American Chemical Society in September 1946. Further work by Nystrom and Brown (6) has shown that this reagent reduces aldehydes, ketones, esters, acid chlorides, anhydrides, and acids to the corresponding alcohols in ether solution at room temperature.

Typical reductions with reducible groups can be represented by the following equations (6):

Aldehydes or Ketones



Esters



Acid Chlorides



Acid Anhydrides



The lithium aluminum complex forms almost instantaneously and precipitates out of the ether solution. Subsequent treatment with water splits the complex into the alcohol, lithium hydroxide, and aluminum hydroxide.

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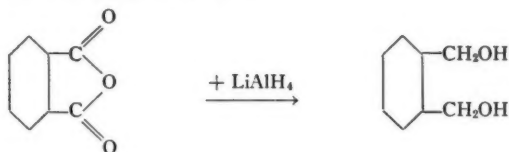
Contribution from the Research Laboratories, Ayerst, McKenna & Harrison, Montreal.

² Present address: Maloney Clinic, Philadelphia 4, Pa.

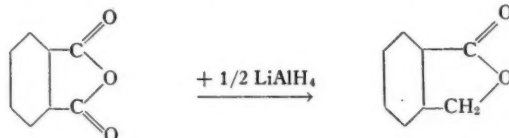
It was thought that because of these properties, lithium aluminum hydride would be a useful reagent in partial reductions such as those mentioned above. In fact, if lithium aluminum hydride is added slowly to an ether solution of the substance to be reduced, the concentration has no chance to build up, since it is used almost immediately upon addition. Moreover, since the complex is insoluble in ether, the most reactive of the reducible groups would be the first and only one to react provided no excess of the reagent is added over the theoretical amount.

The partial reduction of phthalic anhydride to the lactone phthalide was chosen as a model reaction to verify the above hypothesis.

Complete reduction of phthalic anhydride to phthalyl alcohol would require one mole of lithium aluminum hydride.



When one half mole of lithium aluminum hydride is slowly added to one mole of the anhydride, if the above reasoning is correct, the only product will be phthalide:



Whereas if, contrary to the hypothesis, the complex formed by the reaction of one carbonyl group is still reactive, the product should be a mixture of phthalyl alcohol, phthalide, and unreacted phthalic anhydride.

Experimental

Reduction of Phthalic Anhydride to Phthalide

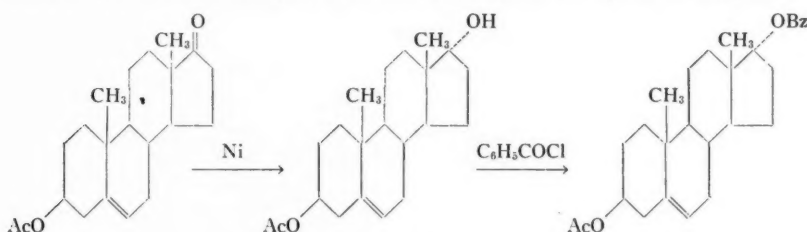
The reduction was done as follows. Phthalic anhydride was dissolved in carefully dried ether and placed in a three-necked flask fitted with a dropping funnel, a stirrer and mercury seal, and a reflux condenser protected by a calcium hydroxide drying tube. An ether solution containing one half mole of lithium aluminum hydride per mole of phthalic anhydride was added dropwise through the funnel, with stirring. When all the reagent had been added, the mixture was cooled in an ice bath, water added, and the mixture was acidified with dilute sulphuric acid to dissolve aluminum hydroxide. The mixture was then transferred to a separatory funnel, the ether phase was separated, and the aqueous phase extracted exhaustively with ether. From the ether, there was obtained a 45% yield of phthalide. No phthalyl alcohol or phthalic anhydride could be identified. It is possible that phthalyl alcohol

remained in the water used to decompose the lithium aluminum hydride complex. However, the result looked promising and no attempt was made to improve the yield.

A similar result was obtained by reacting monomethyl phthalate with three-quarters of a mole of lithium aluminum hydride (one-quarter of a mole being required for the active hydrogen of the free carboxyl group).

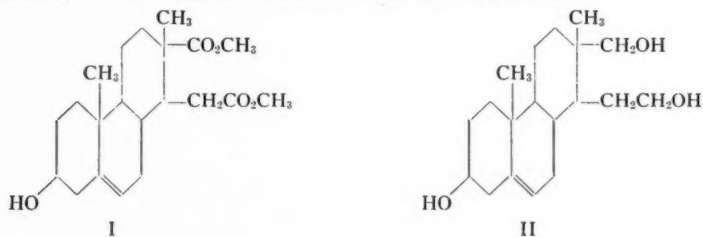
Reduction of Dehydroisoandrosterone Acetate to Δ^5 -androstenediol-3-acetate

This reaction is a step in the synthesis of testosterone from dehydroisoandrosterone. Ruzicka (8) accomplished it by reducing dehydroisoandrosterone acetate with a nickel catalyst, then benzoylating the crude reduction product:



In the authors' hands, with Raney nickel prepared by digestion at 50°C ., the reaction has given yields of about 50%; with lithium aluminum hydride, the reaction was carried out in the manner described above for phthalic anhydride, using a 1 : 4 molar ratio of lithium aluminum hydride to the ester. The crude product obtained by evaporation of the ether extract was benzoylated to pyridine at room temperature and after one recrystallization an 80% yield of Δ^5 -androstenediol-3-acetate-17-benzoate, m.p. 169° to 174°C ., was obtained.

Reduction of Δ^5 -3(β)-hydroxyetiobilienic Acid Dimethyl Ester



Δ^5 -3(β)-hydroxyetiobilienic acid dimethyl ester (I) has been prepared by Wettstein *et al.* (9) and Kuwada (3). In the course of an investigation of the physiological properties of different derivatives of this acid, it was desired to obtain the completely reduced trihydroxy alcohol $\Delta^9,14$ -2,13-dimethyl-7(β)-hydroxy-2-hydroxymethyl-1-hydroxyethyl-dodecahydrophenanthrene (II). Since the ester had to be reduced completely the procedure was modified as follows.

An excess of lithium aluminum hydride in ether solution was placed in the reaction flask and an ether solution of the ester was added at such a rate that the ether kept refluxing. The mixture was refluxed for one-half hour after the addition was complete. After cooling, the complex was decomposed with dilute acid and owing to the low solubility of the product in ether it was extracted with *n*-butanol. The residue obtained by evaporating the solvent *in vacuo* was recrystallized from acetone-hexane; this yielded white crystals melting at 196° to 198° C. (corr.), $(\alpha)_D = -63^\circ$ (alc.). The total yield was 84% of theory. Calc. for $C_{18}H_{32}O_3$, C = 73.98; H = 10.46%. Found: C = 73.80, 74.04; H = 10.76, 10.80%. The tribenzoate was prepared, using benzoyl chloride in pyridine at room temperature. After two recrystallizations from acetone-methanol, the tribenzoate melted at 133° to 136° C. (corr.). Calc. for $C_{40}H_{44}O_6$, C = 77.42; H = 7.10%. Found: C = 77.23, 77.43; H = 7.44, 7.42%. $(\alpha)_D = -14.5^\circ$ (chloroform).

Reduction of Oestrone to α -Oestradiol

Oestrone has been reduced to oestradiol by different methods (4, 5, 10) and the product obtained is generally a mixture of two epimers. Since the α -epimer possesses a much greater physiological activity than the β -epimer (10), it is desirable to use a reduction method that yields principally α -oestradiol. With lithium aluminum hydride, oestrone was used as the acetate, which is appreciably more soluble in ether. With an excess of the reagent as described above for etiobilienic acid ester, it gave a 95% yield of α -oestradiol melting at 173° to 176° C., $(\alpha)_D +74^\circ$.

The absence of β -oestradiol was demonstrated, the method of Carol and Molitor (1) being utilized. These authors have developed a modification of the Kober test to distinguish between α - and β -oestradiol. In the *standard* Kober test, the sample of oestrogen is first heated in boiling water for 20 min. with the reagent, then diluted with an equal amount of water, and heated again for three minutes. These investigators have found that when the 20 min. reaction is done at room temperature (*cold* Kober test), α -oestradiol or oestrone gives very little color upon the final three minute heating, whereas β -oestradiol gives full color development, i.e., same intensity as that given by oestrone or α -oestradiol in the *standard* Kober test. The crude α -oestradiol obtained by lithium aluminum hydride reduction was tested by the *cold* Kober assay and compared with oestrone, β -oestradiol, and a commercial sample of α -oestradiol. The results are given in graphical form in Fig. 1. Obviously, the commercial sample contains appreciable quantities of the β -isomer, whereas the substance obtained by lithium aluminum hydride reduction does not contain more than a trace of β -oestradiol. Since this work has been completed, similar results were reported by Ott and Murray (7, p. 17K) for the reduction of oestrone methyl ether with lithium aluminum hydride.

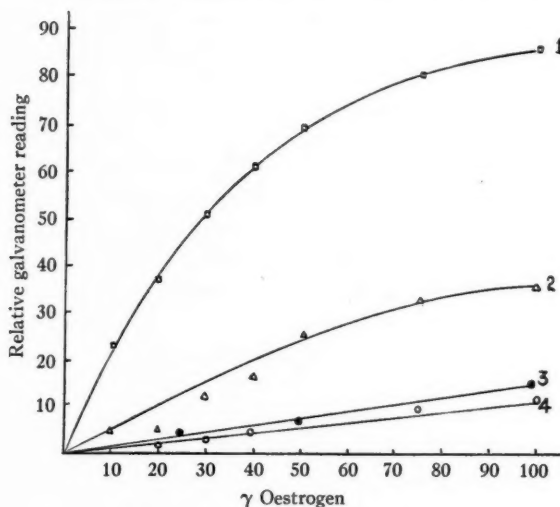


FIG. 1. Cold Kober test on oestrogens.

- 1 (Squares) β -oestradiol
- 2 (Triangles) α -oestradiol (commercial sample)
- 3 (Filled circles) α -oestradiol (LiAlH_4 reduction)
- 4 (Circles) Oestrone.

Acknowledgments

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DETERMINATION OF CELLULOSE IN STRAW HOLOCELLULOSE¹

BY G. A. ADAMS AND A. E. CASTAGNE

Abstract

Hydrolysis of wheat, oat, rye, and flax holocelluloses with 0.5% sulphuric acid for one hour at 100° C. gave a residue that was quantitatively equal to the Cross and Bevan cellulose from the same straws. The hydrolyzed holocellulose residue contained more lignin and less pentosan than the corresponding Cross and Bevan cellulose, but 'pure' cellulose contents (cellulose corrected for ash, lignin, and pentosan) were very similar for both materials; the α -cellulose contents for the two fractions were also equivalent and were slightly higher than the 'pure' cellulose. The α -cellulose contents of the holocellulose were higher than either the Cross and Bevan cellulose or the hydrolyzed holocellulose residue when calculated on the basis of the original straw. The hydrolysis method has the advantage of permitting consecutive determination of holocellulose, cellulose, and α -cellulose on the same sample of straw.

Introduction

Increasing interest in the holocellulose fraction of woods and nonwoody plants has led to the development of several methods for the isolation of this fraction (10, 13, 14, 16). On removing the easily hydrolyzable fraction of spruce holocellulose with dilute acid, Kurth and Ritter (3) obtained a residue that was very similar to Cross and Bevan cellulose. The original procedure was later modified by Van Beckum and Ritter (14) and applied to nine different wood holocelluloses. Increasing the acid strength and hydrolysis time yielded a cellulosic fraction that was quantitatively equivalent to Cross and Bevan cellulose. Further study by Van Beckum and Ritter (15) on aspen, balsam fir, eastern hemlock, jack pine, red spruce, and white spruce showed that holocellulose could be hydrolyzed to a cellulosic fraction comparable in yield to Cross and Bevan cellulose but differing in pentosan content.

While the hydrolysis of holocellulose has been successfully applied to the determination of cellulose in wood, no similar application appears to have been made on cereal straws. Some preliminary experiments in this laboratory showed that the described conditions for hydrolysis when applied to straw holocellulose were too severe. It appeared worthwhile therefore to establish the conditions required for successful applications to straw holocellulose. In addition, a comparison was made between the major constituents of the hydrolyzed holocellulose and of Cross and Bevan cellulose.

Materials and Methods

Clean mature wheat, oat, rye, and flax plants were harvested from experimental plots. The heads were removed but the leafy structures were retained with the straight stem sections. The materials were ground in a Wiley mill

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and then sized in a Rotap screener, using Tyler standard screens. The fraction passing 40 mesh and retained on 60 mesh was used in the subsequent investigations.

Holocellulose was prepared by delignification of the straws with acidified sodium chlorite as previously described (2); Cross and Bevan cellulose content of straw was determined by the Norman and Jenkins hypochlorite method (6). The following methods of analysis were applied to straw, hydrolyzed holocellulose, and Cross and Bevan cellulose: lignin by the 72% sulphuric acid method of Ritter, Seborg, and Mitchell (11); pentosan as furfural determined photocolourimetrically (1); α -cellulose as described by Ritter (9); ash by incinerating at 550° C.; and moisture by drying to constant weight at 100° C. *in vacuo*. Samples that had been oven dried were not used for any subsequent analysis except ash.

Experimental Procedures and Results

Holocellulose was isolated from each of the straws in approximately 50 gm. lots. The yields for these quantities compared very closely with the analytical results from 2.0 gm. samples given in Table I. Flax proved most difficult to

TABLE I
COMPOSITION OF STRAWS, % (DRY BASIS)

	Wheat	Oat	Rye	Flax
Ash	4.67	8.85	4.71	2.68
Lignin (apparent)	23.00	22.26	23.05	21.52
Lignin (ash free)	21.33	19.72	22.20	21.52
Pentosan	27.45	22.67	24.15	21.44
Holocellulose	71.0	69.3	66.0	71.0

delignify and required six chlorite treatments to reduce the lignin to 1.10% (ash free). The ash-free lignin contents of the wheat, oat, and rye holocellulose samples were 2.13%, 2.42%, and 2.40% respectively. These values are considerably higher than those previously reported (1); sample trials showed, however, that the use of unextracted straws compared with extracted straws accounted almost entirely for the difference. The lignin, ash, pentosan, and holocellulose contents of various straws reported in Table I show satisfactory agreement with earlier data (8, 12).

Since a comparison was to be made between Cross and Bevan cellulose and hydrolyzed holocellulose it seemed desirable to prepare both materials from the same starting material, i.e., holocellulose. However, the Norman and Jenkins hypochlorite procedure for the preparation of Cross and Bevan cellulose was much too drastic when applied to holocellulose. A series of experiments using milder procedures was therefore conducted (Table II). Extraction of wheat holocellulose with boiling 3% sodium sulphite produced only a minor reduction in weight. Combination of the sulphite extraction with neutral

TABLE II

YIELDS OF CRUDE CELLULOSE OBTAINED BY CHLORINATION AND SULPHITE EXTRACTION OF WHEAT HOLOCELLULOSE

(Based on dry straw having a Cross and Bevan cellulose content of 56.2%)

Treatment	Yield of cellulose, %	Treatment	Yield of cellulose, %
None	72.0	A BBC	49.3
A	67.4	A C	52.6
A B	51.5	A CC	51.8*
A BB	50.3	A CCC	50.7*

A—Boiled for 20 min. in 3% sodium sulphite solution.

B—Neutral hypochlorite followed by boiling in sulphite.

C—Acidified hypochlorite followed by boiling in sulphite.

* Negative test for lignin.

hypochlorite treatments caused substantial losses in weight, and the residue was about 6% less in weight than the Cross and Bevan cellulose content of the original straw. Application of two neutral and one acid hypochlorite treatments proved to be too severe although the residual material still gave a slight color test for lignin. No acid hypochlorite treatment was found that was mild enough to give a residue corresponding quantitatively to Cross and Bevan cellulose. Since a Cross and Bevan cellulose was not produced by the hypochlorite treatment of holocellulose, it was prepared directly from straw by the Norman and Jenkins method (6); the yield was 56.2%. This value was used as a standard of comparison for cellulose yields from the hydrolysis of holocellulose.

Hydrolysis of Wheat Holocellulose

The main factors affecting the mild hydrolysis of holocellulose to a residue equivalent to Cross and Bevan cellulose include: concentration of acid, time of hydrolysis, and temperature. A standard hydrolysis temperature of 100° C. was adopted. Preliminary trials showed that a boiling water bath gave more consistent results than direct heating. Only sulphuric acid was considered since its nonvolatility is advantageous for long refluxing periods. Concentrations of acid of 1.3% were shown to be unsuitable in preliminary experiments and only lower concentrations were investigated further. Hydrolysis times of one and two hours were studied.

For the following experiments, wheat straw holocellulose was prepared by three acid chlorite treatments. Only air dried samples were used for the proposed cellulose determination. The procedure was as follows. Two grams of the holocellulose was accurately weighed and transferred to a 500 ml. Erlenmeyer flask, placed in a boiling water bath, and 200 ml. of boiling sulphuric acid solution of known concentration by weight added. The solution was then heated under reflux for a fixed period of time. Care was taken to prevent dry material from collecting on the sides of the flask and the water

level in the bath was kept above that of the solution in the flask. At the end of the heating period the solution was filtered at once in a sintered glass crucible (Porosity C) and washed with hot water until the washings were neutral to litmus. Water was further removed by a successive wash with 25 ml. of ethanol and 25 ml. of ether; after a short period of air drying the material was dried to constant weight *in vacuo* at 100° C. The yield of hydrolyzed holocellulose was calculated on the basis of air dry straw. In addition to the above experiments using acid, samples of holocellulose were subjected to a similar treatment with distilled water. The results of the various hydrolyses are given in Table III. The two hour treatments with various

TABLE III

HYDROLYSIS OF WHEAT HOLOCELLULOSE WITH DILUTE SULPHURIC ACID
(Holocellulose yield = 71.0% of original straw; Cross and Bevan cellulose content of straw = 56.2%, dry basis)

Strength of acid, % (wt.)	Yield of holocellulose residue, % (based on dry straw)	
	1 hr. heating	2 hr. heating
0	67.2	66.1
0.4	58.5	51.4
0.5	56.3	51.0
0.6	55.6	50.5
0.7	54.5	50.4
0.8	54.0	50.4
0.9	53.5	50.3
1.0		49.6
1.1		49.5
1.2		49.5

strengths of acid were all too drastic, the yield of hydrolyzed holocellulose being less than the Cross and Bevan cellulose. Hydrolysis with 0.5% sulphuric acid for one hour gave a yield of cellulose that was equal in amount to Cross and Bevan cellulose. These conditions were chosen therefore for further investigations of the wheat hydrolyzed fraction. A systematic study of the hydrolysis conditions was limited to wheat holocellulose since trial experiments indicated similar effects on the corresponding fractions from the other straws.

Hydrolysis of wheat holocellulose with acids of the various strengths for a two-hour period yielded a residue that constituted about 50% of the weight of the straw. The uniformity of yield suggests a constant cellulose fraction that merits further investigation. In yield, it approximates more closely to Cross and Bevan cellulose than to α -cellulose. Moreover, Table II shows that a similar residue was obtained by application of the hypochlorite treatments (acid and neutral) to wheat holocellulose.

Hydrolysis of Dried and Undried Holocellulose

In a routine analysis of straw for holocellulose and cellulose the most timesaving procedure is as follows: four weighed samples of straw are delignified to holocellulose with acid chlorite; two are dried to determine the holocellulose yield and two are thoroughly washed with cold 0.5% sulphuric acid and then hydrolyzed to yield the cellulose fraction. However, in most of the present investigation it was more convenient to prepare a quantity of each holocellulose in the air dried state and use it for the acid hydrolysis experiments and subsequent analyses. The following data show that the same cellulose and α -cellulose yields can be obtained from both dried and undried samples of holocellulose:

	Crude cellulose, % (dry straw basis)	α -cellulose, % (dry straw basis)
Wheat holocellulose (dry)	56.2	38.7
Wheat holocellulose (undried)	55.9	38.3

Comparison of Yields and Composition of Cross and Bevan Cellulose and Hydrolyzed Holocellulose

Cross and Bevan cellulose and hydrolyzed holocellulose were prepared from straw in 10 gm. lots by methods already described. The samples were air dried. The precision of the Cross and Bevan determination was good, the deviation from the mean being about 0.1%; that of the hydrolyzed holocellulose was about 0.3%. Much depends on the precision of the preceding holocellulose preparation; if overchlorination has taken place the results are less precise. The composition of the two fractions is shown in Table IV. The yields of Cross and Bevan cellulose and hydrolyzed holocellulose agree very closely with each other for each straw. Correction of the various fractions for ash content showed that the celluloses prepared from hydrolyzed wheat, oats, and rye holocellulose contained considerably more ash than their corresponding Cross and Bevan celluloses. The ash content of flax fractions proved to be an exception, Cross and Bevan cellulose and hydrolyzed holocellulose containing 0.86% and 0.44% respectively. The ash content of wheat, oat, and rye holocelluloses was higher than that of the straw from which holocelluloses were prepared (2) and apparently the treatment with dilute sulphuric acid did not reduce it.

The apparent lignins in the crude Cross and Bevan celluloses were relatively low, the order of amount being in general agreement with reported values for straws (5, 6). On an ash-free basis the values for wheat and oat lignins were reduced substantially while rye and flax lignins were ash free. The apparent lignins of the hydrolyzed holocellulose were quite high for wheat, oats, and rye. However, their ash content is high, so that the ash-free lignin contents become a better basis for comparison. Nevertheless, they are still more than

TABLE IV

COMPOSITION (%) OF CROSS AND BEVAN CELLULOSE AND HYDROLYZED HOLOCELLULOSE FROM VARIOUS STRAWS

	Wheat	Oat	Rye	Flax
<i>Cross and Bevan cellulose</i>				
Crude cellulose	56.2	52.0	55.6	52.8
Cellulose corrected for ash	55.6	51.5	55.1	52.4
Pentosan in crude cellulose	31.9	29.3	29.5	23.9
Lignin (apparent) in crude cellulose	1.30	2.28	1.60	0.87
Lignin (ash-free) in crude cellulose	1.16	1.97	1.60	0.87
'Pure' cellulose in original straw	36.9	35.2	37.8	39.2
α -Cellulose in crude cellulose	69.5	74.9	71.8	72.8
<i>Hydrolyzed holocellulose</i>				
Crude cellulose	56.1	52.7	55.2	52.3
Cellulose corrected for ash	53.9	49.8	54.1	52.0
Pentosan in crude cellulose	25.6	21.5	21.4	18.2
Lignin (apparent) in crude cellulose	4.48	7.47	5.08	1.48
Lignin(ash-free) in crude cellulose	2.84	3.64	3.65	1.48
'Pure' cellulose in original straw	37.3	34.8	39.5	44.4
α -Cellulose in crude cellulose	70.8	74.4	71.4	68.4

twice as great as the lignin in the Cross and Bevan fractions. In the acid hydrolysis step there is no delignification, and the recovered fraction contains all the lignin present in the holocellulose. Since the residual lignin in holocellulose was of the order of 2 to 3% the amount in the hydrolyzed fraction was proportionately higher. Again the flax lignin was an exception. It was ash-free and while greater in amount than in the corresponding Cross and Bevan fractions it was still relatively low. This low value was due to the six chlorite treatments of the flax straw in the holocellulose preparation that have been found necessary to obtain a lignin content comparable with those of the other holocelluloses (1).

For all straws, the pentosan content of the Cross and Bevan cellulose is approximately 25% higher than that of the hydrolyzed holocellulose. The chlorination procedure used in the preparation of Cross and Bevan cellulose removes not only the lignin but most of the polyuronide hemicelluloses, the cellulosan fraction being largely unchanged (15). The hemicellulose fractions are left almost intact in the preparation of straw holocellulose by acid chlorite. The lower pentosan content of hydrolyzed holocellulose compared with Cross and Bevan cellulose suggests that, in the former, cellulosan material has been removed in addition to the easily hydrolyzed polyuronides. In a comparison of the components of Cross and Bevan cellulose and hydrolyzed holocellulose from various woods, Van Beckum and Ritter found more pentosan in the Cross and Bevan cellulose from aspen while the comparable softwood fractions

had a higher pentosan content in the hydrolyzed holocellulose (14). These observations may be accounted for by the close similarity in chemical composition of hardwoods and cereal straws.

The crude celluloses obtained by both the chlorination of the straws and hydrolysis of their holocellulose have similar α -cellulose contents. The somewhat lower content of α -cellulose in hydrolyzed flax holocellulose indicated degradation during the preparation. While the yield of crude flax cellulose was the same for both fractions it is apparent that the hydrolyzed material was readily susceptible to the solvent action of 17.5% sodium hydroxide. The extensive delignification treatments of the original flax straw with sodium chlorite undoubtedly contributed to this susceptibility. The recovery of equal yields of α -cellulose from Cross and Bevan cellulose and hydrolyzed cellulose shows the feasibility of determining α -cellulose directly from hydrolyzed holocellulose. The determination of holocellulose, cellulose, and α -cellulose can therefore be made in succession from the same sample of straw.

The 'pure' cellulose contents of the fractions are values calculated by correcting crude cellulose for ash, pentosan, and lignin and are expressed on the basis of the original straw. The pure cellulose contents in the Cross and Bevan cellulose and in the hydrolyzed holocellulose are similar for wheat, oat, and rye straws. Flax shows a higher yield in the hydrolyzed holocellulose. The α -cellulose contents of both Cross and Bevan and hydrolyzed holocellulose are higher than their pure cellulose contents. This is probably due to the inclusion of pentosan material in the α -cellulose. Lewis (4) reports that fibrous residues contain as much as 8% more of α -cellulose than of pure cellulose although the reverse is true of wood.

TABLE V
 α -CELLULOSE CONTENT OF VARIOUS CELLULOSE FRACTIONS, %
(Calculated on basis of dry straw)

	Wheat	Oat	Rye	Flax
Holocellulose	40.0	40.4	40.1	39.0
Cross and Bevan cellulose	39.1	39.0	39.9	38.4
Hydrolyzed holocellulose	38.7	38.6	40.0	35.2

In Table V, α -cellulose contents of the crude cellulose fractions were calculated on the basis of original straw. The α -cellulose contents of the holocelluloses for all straws were slightly higher than in either of the other fractions. It is apparent that acid hydrolysis of holocellulose caused a loss in α -cellulose. Flax holocellulose was affected more than the others. The close agreement between the yields of α -cellulose from hydrolyzed holocellulose and Cross and Bevan cellulose indicate that preparation of the latter also involves a small loss of α -cellulose. The differences in α -cellulose contents between the various fractions are similar to those found by Van Beckum and Ritter for

aspen wood (14); the same authors found considerably more α -cellulose in softwood holocellulose than in the corresponding Cross and Bevan and hydrolyzed fractions.

The determination of Cross and Bevan cellulose is empirical in nature and depends on the number and severity of treatments required for delignification. Deviation from the prescribed procedures will cause the isolated residues to vary both qualitatively and quantitatively. The method under the best conditions provides a good but by no means absolute separation of the polyuronides and cellulose. Mild acid hydrolyses of holocelluloses removed not only the polyuronides, which are solubilized in the Cross and Bevan cellulose method, but also caused a mild attack on the cellulose as shown by the lower pentosan content of the cellulosic residue.

Other proposed methods of cellulose determination including the ethanolamine (8) and the ethanol - nitric acid procedures (5) produce similar effects. Recovery of equal yields of α -cellulose from the Cross and Bevan cellulose and the hydrolyzed holocellulose indicated that the main cellulosic component was preserved to the same extent by both methods of analysis. The chlorination method yields useful though empirical information, but the production of a similar fraction by hydrolysis of holocellulose provides an easier and more rapid method of obtaining the same data. The hydrolysis method has the added advantage of providing means of determining holocellulose, cellulose, and α -cellulose in succession on the same sample of straw. Furthermore, the method is readily applicable to large scale preparation of the same materials.

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DETERMINATION OF LIGNIN IN CEREAL STRAWS¹

By G. A. ADAMS AND A. E. CASTAGNE

Abstract

Determination of lignin in wheat, oat, barley, and rye straw with 64%, 68%, 72%, 76%, and 80% (wt.) sulphuric acid showed that a minimum yield of lignin containing a maximum amount of methoxyl was obtained with the 72% sulphuric acid. In this respect, cereal straws differ from deciduous woods for which 72% sulphuric acid lignin determinations are unsuitable. Comparison of lignin determinations made with 72% sulphuric acid and with 42% fuming hydrochloric acid, on the same straws, after pretreatment with 1.0% hydrochloric acid, showed that the former method preserves a larger proportion of the methoxyl groups. Both methods recovered more total lignin methoxyl from untreated than from treated straws, with the sulphuric acid retaining the larger amount. It is concluded that lignin determination with 72% sulphuric acid on straws untreated with dilute acid yields a residue most representative of the 'true' lignin content, based on the criterion of maximum methoxyl recovery.

Introduction

Of the many methods proposed for the determination of lignin in fibrous plant materials, the 72% sulphuric acid and the 42% fuming hydrochloric acid procedures have been most widely used. Many modifications of time and temperature of digestion have been introduced to minimize the effect of these strong acids on the carbohydrate fraction. Until recently little attention was paid to the concentration of the acid because 72% sulphuric acid had become the generally accepted reagent, although Klason, who developed the method, did not claim general suitability of this concentration for all woody materials (8, 9). Pretreatment of the wood or plant material to increase the accuracy of the lignin determination has been extensively investigated. Waksman and Cordon have pointed out that a short period of dilute acid treatment, sufficient to remove easily hydrolyzable carbohydrates prior to the preparation of acid lignin, is required (18). Norman and Jenkins have also studied the problem in detail and recommend a brief dilute acid hydrolysis treatment before determining lignin (12). Phillips and Goss also accept the principle of acid pretreatment (13). On the other hand, Bamford and Campbell found that xylose, fructose, and sucrose when treated with 72% sulphuric acid at 10° C. for five hours yielded negligible quantities of insoluble residues and were therefore unlikely to interfere seriously in lignin determinations (2). Pretreatment of the plant or wood material with either hot water or dilute acid is rejected by Cohen and Harris on the grounds that both have solvent action on the lignin (5). Ploetz states that the pretreatment of woods is no longer recommended, as dilute acids can remove a portion of the lignin (14). In reviewing the evidence Norman points out that the present information

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does not warrant immediate rejection of the acid hydrolysis pretreatment although doubts as to its suitability are raised (11).

It has been usually considered that the proper concentration of sulphuric acid for lignin determination is that which gives a minimum yield of lignin. If the concentration is too low the polysaccharides remain in the lignin residue; if too high, humic substances are formed by the action of the acid on the carbohydrates and are recovered with the lignin. For many woods, 72% sulphuric acid produces a minimum yield of lignin. However, Freudenberg and Ploetz (6) have found this criterion of minimum yield to fail with linden (*Itelia tomentosa*) in a study covering 62.5 to 80.5% sulphuric acid. With red beech (*Fagus silvatica*) and elder (*Sambucus nigra*) the optimum concentration of acid was found to be 66.5%. They proposed therefore that the term "sulphuric acid lignin" be substituted for "Klason lignin" (72% sulphuric acid lignin) and that the product be characterized by maximum methoxyl content. In a similar study Ploetz (14) found that 66.5 to 75% sulphuric acid gave a constant yield of spruce lignin although the methoxyl reached a maximum only at 75% acid. Some deciduous woods yielded minimum lignin containing maximum methoxyl with 66.5% sulphuric acid. Brauns (3) has also pointed out that the discrepancy between calculated and found methoxyl content for maple lignin is due in part to the inaccuracy of the lignin methods.

The unsuitability of the 72% sulphuric acid method for lignin in deciduous wood raises the question whether or not the same method is applicable to fibrous plant materials, which are quite similar in chemical composition to the hardwoods. While the 42% fuming hydrochloric acid method is frequently used for fibrous plants, a great many lignin determinations on these materials are done by the sulphuric acid method because of its simplicity and convenience.

The purpose of the present study was to investigate the effect of a range of sulphuric acid concentrations (64 to 80%) on the yield of lignin from extracted straws. The methoxyl content of the various isolated lignins was determined, as well as the total reducing sugar and pentosans in the hydrolyzates. For comparison, the lignins were determined in the same straws by the 42% fuming hydrochloric acid method. The effect of pretreatment with dilute acid on lignin yield from extracted straws by both methods of determination was included in the study.

Materials and Methods

Clean mature wheat, oat, barley, and rye straws, harvested by hand from experimental plots, were used in the study. The straws were cut in about 1 in. lengths and then milled in a laboratory hammer mill. The ground material was then sized with standard screens on a Ro-tap screener and the -40 to +60 mesh size retained for use in subsequent experiments. All straws were extracted with 1 : 2 alcohol-benzene and hot water according to the standard TAPPI procedure (16). The extracted straw was analyzed for moisture by

drying to constant weight in a vacuum oven at 100° C. Lignin was determined by the sulphuric acid method described by Ritter, Seborg, and Mitchell (15) and by the standard 42% fuming hydrochloric acid method (1). Pentosans were determined as furfural after distillation with 12% hydrochloric acid according to Hughes and Acree (7), total reducing sugars by the procedure of Underkofler *et al.* (17), methoxyl by the Viebock and Schwappach volumetric procedure as modified by Clark (4, p. 68), and ash by incinerating at 550° C. The sulphuric acid solutions for lignin determinations were prepared on a percentage by weight basis, titrated with standard alkali, and adjusted to $\pm 0.1\%$.

Experimental Procedures and Results

Effect of Sulphuric Acid Concentration on Lignin Determination

Since the concentration of sulphuric acid was the factor being investigated, other factors that influence the determination were standardized as outlined by Ritter *et al.* (15). Slightly larger samples of extracted straw were used. The concentrations of strong sulphuric acid used were 64.0%, 68.0%, 72.0%, 76.0%, and 80.0%. After hydrolysis, the mixture was allowed to cool to room temperature, filtered on a sintered glass crucible (M porosity), washed free of acid with hot water, air-dried overnight, and then brought to constant weight in a vacuum oven at 100° C. Six separate lignin determinations were made on each straw at each concentration of acid. Agreement between individual analyses showed that it was possible to bulk the samples, and the composite sample was used for ash and methoxyl determinations. The solutions from the dilute acid hydrolyses were combined with the washings from the lignin separation, made to volume, and analyzed for total reducing sugars and furfural. Two of the six test solutions were used for these analyses. The results of the various analyses are given in the tables as follows: Table I, wheat; Table II, oat; Table III, barley; and Table IV, rye.

TABLE I

LIGNIN AND SUGAR DETERMINATIONS (%) IN WHEAT STRAW HYDROLYZED WITH SULPHURIC ACID OF VARIOUS STRENGTHS

(Straw extracted with alcohol-benzene and hot water, dry wt. basis)

Sulphuric acid	Lignin residue			Hydrolyzate	
	Lignin	Ash-free lignin	Methoxyl in ash-free lignin	Total reducing sugars	Pentosans as furfural
64.0	51.08	49.40	5.1	41.16	20.12
68.0	29.50	27.98	11.9	63.70	20.22
72.0	20.75	19.18	15.2	75.09	20.75
76.0	21.10	19.53	15.0	74.06	19.65
80.0	21.52	19.90	14.1	72.31	19.76

The lignin determination in wheat straw showed that 64% sulphuric acid was insufficient to digest the cellulosic material completely. Somewhat stronger acid solutions dissolved the cellulosic material but it was reprecipitated on dilution of the acid to 3%, and subsequent hydrolyses did not render this material completely soluble. As increasing concentrations of acid were used the lignin value passed through a minimum at 72% acid. The methoxyl content reached a maximum at 72% concentration and accordingly satisfied the criterion of Freudenberg and Ploetz (6), who claim that the most representative lignin has a maximum methoxyl content. The lignin and methoxyl values obtained with less than 72% acid represent contamination with cellulosic residues. Above 72% acid concentration, the lignin content again increases owing to combination with humic substances produced by the action of strong acids on the sugar residues. The lower methoxyl figures, particularly in the 80% acid lignin, may be attributed to admixture with humic substances low in methoxyl content. The change in sugar content of the hydrolyzate supported this view. The sugars reach a maximum with 72% acid. Below 72% there is incomplete hydrolysis of the carbohydrates and above 72% sugars are humidified by the acid and condense with the lignin. There is considerable evidence in the literature to show that such reactions occur (10, p. 169). The relatively small change in the furfural values indicated that the pentose sugars were little affected by the acid treatment. The high pentose content with the lowest concentration of acid showed that the hemi-cellulose fraction was readily hydrolyzed. The sharp reduction in pentose content of the hydrolysate with 75% and 80% acid as reported by Freudenberg and Ploetz (6) for pine, linden, beach, and elder wood was not found for straws, but the two-day treatment with acid used by these investigators may have caused considerably more decomposition of the sugars.

The results for oat straw as given in Table II show, in general, that it behaved similarly to wheat straw. With increasing acid concentrations the lignin yield passed through a minimum with 72% acid. The maximum

TABLE II

LIGNIN AND SUGAR DETERMINATIONS (%) IN OAT STRAWS HYDROLYZED WITH SULPHURIC ACID OF VARIOUS STRENGTHS

(Straw extracted with alcohol-benzene and hot water, dry wt. basis)

Sulphuric acid	Lignin residue			Hydrolyzate	
	Lignin	Ash-free lignin	Methoxyl in ash-free lignin	Total reducing sugars	Pentosans as furfural
64.0	50.12	49.41	4.8	41.81	20.84
68.0	30.78	30.11	8.9	64.90	20.74
72.0	18.78	18.06	13.7	74.28	21.76
76.0	19.04	18.36	13.8	73.36	20.40
80.0	19.53	18.86	13.1	71.70	20.04

methoxyl content in the lignin occurred with 76% acid but the difference from the 72% acid lignin was negligible. The maximum sugar and pentosan contents occur at the 72% acid level and indicate a minimum combination of sugar residues with lignin.

In Tables III and IV, the results for barley and rye straws show further that 72% sulphuric acid gives a minimum yield of lignin. For barley straw

TABLE III

LIGNIN AND SUGAR DETERMINATIONS (%) IN BARLEY STRAW HYDROLYZED WITH SULPHURIC ACID OF VARIOUS STRENGTHS

(Straw extracted with alcohol-benzene and hot water, dry wt. basis)

Sulphuric acid	Lignin residue			Hydrolyzate	
	Lignin	Ash-free lignin	Methoxyl in ash-free lignin	Total reducing sugars	Pentosans as furfural
64.0	46.37	45.89	5.2	46.77	20.97
68.0	35.23	34.75	7.6	61.32	20.69
72.0	17.40	16.83	14.8	73.73	20.89
76.0	17.63	17.06	14.3	77.36	21.34
80.0	18.08	17.51	14.1	73.36	18.75

TABLE IV

LIGNIN AND SUGAR DETERMINATIONS (%) IN RYE STRAW HYDROLYZED WITH SULPHURIC ACID OF VARIOUS STRENGTHS

(Straw extracted with alcohol-benzene and hot water, dry wt. basis)

Sulphuric acid	Lignin residue			Hydrolyzate	
	Lignin	Ash-free lignin	Methoxyl in ash-free lignin	Total reducing sugars	Pentosans as furfural
64.0	48.71	47.95	6.1	48.27	19.96
68.0	35.12	34.40	8.6	57.01	20.08
72.0	19.48	18.79	15.7	71.44	20.07
76.0	19.65	18.98	15.7	73.15	20.36
80.0	20.32	19.56	15.6	72.91	17.60

the maximum methoxyl content of the lignin occurs with 72% acid although the total sugar and furfural figures were slightly higher with 76% acid. While rye straw gave the lowest lignin yield with 72% acid the methoxyl content of the 76% acid lignin was slightly higher. The total sugar and pentosan contents of the hydrolyzate were at a maximum with 76% acid. These data when taken in conjunction with the small differences between the lignin yield with 72% and 76% acid lead to the conclusion that acid concentrations covering this range would be equally satisfactory for determining lignin in rye straw.

It is apparent that the usually employed 72% sulphuric acid gives satisfactory lignin values with cereal straws. In this respect cereal straws resemble the coniferous woods and differ from the deciduous woods.

Comparison of Lignin Determinations by Two Methods on Straws Pretreated with Dilute Acid

Although it has been shown in the foregoing experiments that 72% was the most satisfactory concentration of sulphuric acid for lignin determination in the cereal straws examined, it seemed worthwhile to compare this method with the 42% hydrochloric acid method, which is a standard procedure for plant materials (1). Both acids are known to form huminlike products with carbohydrates although Phillips and Goss (13) claim that the disturbance caused by hydrochloric acid is less—an observation that Norman attributes to the low temperatures that must be used (10, p. 177). Phillips found about 1% less lignin in extracted wheat straw with the hydrochloric acid method although the methoxyl contents of lignins determined by 72% sulphuric acid and 42% hydrochloric acid were almost the same.

Since the official 42% hydrochloric acid method is applied to extracted straws pretreated with 1% hydrochloric acid, the 72% sulphuric acid procedure was used on similarly prepared straw. Extracted wheat, oat, barley, and rye straws were analyzed by both procedures. Methoxyl and ash contents were determined on the isolated lignins. The results are given in Table V.

The 1% hydrochloric acid treatment removed about 35% of the dry substance from all straws. The sulphuric acid lignins were higher than the hydrochloric acid lignins for wheat, oat, barley, and rye straw by 1.28%,

TABLE V

COMPARISON OF LIGNIN DETERMINATIONS (%) IN STRAWS TREATED WITH 1% HYDROCHLORIC ACID, BY THE 72% SULPHURIC ACID METHOD, AND BY THE 42% FUMING HYDROCHLORIC ACID METHOD

(Straw extracted with alcohol-benzene and hot water, dry wt. basis)

Determinations	Wheat straw		Oat straw		Barley straw		Rye straw	
	H ₂ SO ₄	HCl	H ₂ SO ₄	HCl	H ₂ SO ₄	HCl	H ₂ SO ₄	HCl
Yield of treated straws	66.2	65.9	64.7	65.0	63.4	63.9	65.6	65.5
Lignin	25.69	24.11	23.69	22.00	22.17	20.72	23.09	21.91
Ash-free lignin	23.63	21.79	22.69	20.91	21.18	19.76	22.12	20.83
Methoxyl in ash-free lignin	16.3	16.1	14.9	15.0	15.6	15.4	17.3	17.3
Ash-free lignin in untreated straws	15.63	14.35	14.68	13.59	13.42	12.62	14.52	13.65
Lignin methoxyl in untreated straws	2.55	2.31	2.19	2.04	2.09	1.94	2.51	2.36

1.09%, 0.80%, and 0.87% respectively (ash-free basis). The ash content in the wheat straw lignins was about twice that of the other straws. The methoxyl contents of the lignins isolated by the two methods agreed closely with each other for all straws. The results reported for wheat straw lignin are very similar to those found by Phillips and Goss (13). If one applies the criterion that a minimal lignin value is the most representative of the true lignin content then the hydrochloric acid method would be chosen. However if the criterion of Freudenberg and Ploetz, that the maximum methoxyl is the best indication of 'true' lignin, is accepted, then the sulphuric acid method would be chosen since the calculated lignin methoxyl (last line Table V) shows that the maximum methoxyl was contained in the sulphuric acid lignin. It is reasonable therefore to conclude that the 72% sulphuric acid method is preferable to the hydrochloric acid method, which gives a lower total lignin methoxyl content.

Comparison of Lignin Determinations on Untreated Straw

Since the usefulness of dilute acid treatment of cellulosic plant materials prior to lignin determination had not been clearly demonstrated, it seemed advisable to compare the two methods of lignin determination on straws that had not been treated with acid. The 72% sulphuric acid and 42% hydrochloric acid methods were therefore applied to wheat, oat, barley, and rye straws, after alcohol-benzene and hot water extraction. The results are shown in Table VI.

TABLE VI

COMPARISON OF LIGNIN DETERMINATIONS (%) IN STRAW BY THE 72% SULPHURIC ACID METHOD AND THE FUMING HYDROCHLORIC ACID METHOD

(Straw extracted with alcohol-benzene and hot water, dry wt. basis)

Determinations	Wheat straw		Oat straw		Barley straw		Rye straw	
	H ₂ SO ₄	HCl	H ₂ SO ₄	HCl	H ₂ SO ₄	HCl	H ₂ SO ₄	HCl
Lignin	20.75	18.51	18.78	16.39	17.40	15.20	19.48	16.30
Ash-free lignin	19.18	17.03	18.04	15.60	16.80	14.62	18.82	15.62
Methoxyl in ash-free lignin	15.2	15.3	13.7	14.1	14.8	15.0	15.6	16.7
Lignin methoxyl in straw	2.91	2.60	2.47	2.20	2.49	2.19	2.94	2.61

The sulphuric acid lignins (ash-free basis) were 2 to 3% higher than the hydrochloric acid lignins. The methoxyl contents in the lignins isolated by both methods were very similar, with the exception of rye straw. Although hydrochloric acid yielded a minimum amount of lignin with a slightly higher methoxyl content than the sulphuric acid lignin, calculation of the total methoxyl isolated in the lignin residues from wheat shows that $2.91 - 2.60 =$

0.31% more methoxyl was contained in the sulphuric acid lignin. If it is assumed that the higher yield of sulphuric acid lignin ($19.18 - 17.03 = 2.15\%$) was due to the presence of humified carbohydrate, then this excess material would have to contain $\frac{0.31}{2.15} \times 100 = 14.4\%$ methoxyl—an invalid assumption, since it is known that the carbohydrate fraction contains only a small amount of methoxyl. The calculated methoxy content indicates that this material is closely related to lignin. Similar calculations applied to oat, barley, and rye lignins show that the sulphuric acid lignin cannot contain appreciable quantities of carbohydrate residues. On the basis of these results on untreated straws, it is reasonable to conclude that 72% sulphuric acid gives a more truly representative lignin than fuming hydrochloric acid.

Comparison of the data in Tables V and VI shows that the lignins determined by both methods are lower in all straws pretreated with dilute acid, and that pretreatment with acid affected the sulphuric acid method more than the hydrochloric acid method. The methoxyl of the lignins from acid treated straw was higher than from untreated straw. The lower lignin yield from acid treated straws is attributed by Phillips (13) to the removal of interfering substances by the dilute acid. However, if the same calculation as in the preceding section is applied, it is evident that this is not so. Untreated wheat straw contained $19.18 - 15.63 = 3.55\%$ more 'lignin' than the treated straw. However, since the total lignin methoxyl was $2.91 - 2.55 = 0.36\%$ higher in the lignin from untreated straw, then this excess 'lignin' must have a calculated methoxyl content of $\frac{0.36}{3.55} \times 100 = 10.15\%$. Again the value is too high to be attributed to humified carbohydrate material. The same calculation applied to hydrochloric acid lignin from treated and untreated wheat straw shows that untreated straw yields a lignin residue containing more total methoxyl. The increase over that of treated straws cannot be due to humified carbohydrate material. Similar data leading to the same conclusion can be calculated for the other straws. These results lend indirect support to the evidence of Cohen and Harris (5) that a dilute acid pretreatment removes a portion of the lignin.

The proposal of Freudenberg and Ploetz (6) that the criterion for lignin determinations should be a residue with a maximum methoxyl content appears to be justified. It has been shown that the determination of lignin in untreated straws with 72% sulphuric acid more nearly satisfies the requirements of 'true' total lignin than determination with 42% hydrochloric acid. It is also worth noting that the sulphuric acid method is faster and more convenient and avoids the unpleasantness associated with the use of fuming hydrochloric acid.

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DISTRIBUTION OF METHOXYL AND URONIC ACID GROUPS IN CEREAL STRAWS¹

BY G. A. ADAMS AND A. E. CASTAGNE

Abstract

The distribution of methoxyl and uronic acid groups in wheat and oat straw has been investigated in the following successive fractions: extractive-free straw, pectin-free straw, holocellulose, hemicellulose "A", hemicellulose "B", and " α -cellulose" residue. About 18% of the total methoxyl content of extractive-free straws was found in the holocellulose fraction, the remainder being associated with the lignin portion. Methoxyl groups in the hemicellulose fractions and residues accounted for only about 70% of those present in the holocelluloses. No glycosidic methoxyls were found and the amount of ester methoxyl was too small to account for losses by saponification. The conclusion was reached that some of the ether methoxyls were cleaved during the alkali extraction of the holocelluloses. Methoxyl values in the α -cellulose residues as obtained by the Zeisel method were considerably greater than those of the trimethylamine procedure. Uronic acid groups of the extractive-free straw were almost quantitatively accounted for in the various fractions. The molar ratio $\frac{\text{uronic acid anhydride}}{\text{methoxyl}}$, which was less than unity in the holocelluloses, increased to greater than unity in the hemicelluloses A and B of both straws.

Introduction

The presence of methoxyl groups in the cellulosic portion of wood and straws has been clearly established. Ritter and Barbour found approximately 88% and 77% of the methoxyl of red wood and white oak respectively associated with lignin, the remainder being distributed between extraneous material and holocellulose (18). A wheat straw holocellulose isolated by Schenck and Kurth contained 19% of the total methoxyl of the extractive-free straw (21). Kurth and Ritter prepared holocellulose fractions containing 12% of the total methoxyl from extractive-free spruce wood (12). The distribution of the methoxyl groups in various hemicellulosic fractions prepared by alkaline treatment of woods and fibrous plants has received considerable attention. Most of the methods of hemicellulose isolation are empirical and represent a great diversity of alkaline extractions. Many have followed the well known procedure of Norris and Preece (16). While the presence of lignin in the fractions has complicated the interpretation of the methoxyl values, there seems to be general agreement that the methoxyl groups are attached to uronic acids in methoxyhexuronic acid units. In mesquite wood hemicelluloses Sands and Nutter found approximately one methoxyl group per uronic acid molecule (20). On further purification by chlorination, definitely less than one methoxyl per uronic acid was obtained. A molar ratio of uronic acid to methoxyl of 1.6 was found by Sitch (22) for an ethylenediamine soluble fraction of white birch.

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* The present investigation was concerned with the distribution of methoxyl groups and uronic acids in various fractions of extractive-free wheat and oat straws. Holocellulose preparations were used to avoid the interference of lignin in the alkali soluble fractions and residues. No attempt was made to separate a variety of hemicellulose fractions but a procedure was used that yields broad hemicellulosic fractions quantitatively. It was hoped, in this way, to follow the preservation and distribution of the methoxyl and uronic acids in empirical but quantitative fractions.

Materials and Methods

Clean mature wheat and oat straw was cut into 1 in. lengths and then ground in a Raymond laboratory pulverizer. The fraction that passed through a 40 mesh screen and was retained on 60 mesh was used in the subsequent investigations. The material was extracted with 1:2 alcohol-benzene and hot water according to the standard Tappi method (23). This extractive-free straw was considered as the starting material for this study. Pectin substances were extracted with 0.5% ammonium oxalate by three separate four-hour treatments at 85° C. and the residue was thoroughly washed with water at 40° C. The ammonium oxalate extract was concentrated to one-quarter volume and poured into acidified ethanol to precipitate the pectin. Holocellulose was prepared by the acid chlorite procedure previously described (1). Hemicelluloses were isolated from the holocellulose according to Wise *et al.* (27). Lignin determinations were made by the 72% sulphuric acid method (19). Total methoxyl was determined by Clark's modification of the Viebock and Schwappach procedure (6, p. 68); ester methoxyl by von Fellenberg's method (9, p. 68) and glycosidic methoxyl according to Hoffpauir and Reeves (11) except that the formaldehyde was determined with chromotropic acid instead of Schiff's reagent. Uronic acid was determined by a modification of the method of Dickson *et al.* (8) in which the carbon dioxide produced by decarboxylation was removed by a stream of carbon-dioxide-free nitrogen in an all-glass apparatus. Moisture was determined by drying to constant weight at 100° C. in a vacuum oven; ash by incinerating at 550° C.

Experimental Procedures and Results

Composition of Extractive-free and Pectin-free Straws

The lignin and methoxyl contents of extractive-free wheat and oat straw before and after extraction of pectin by 0.5% ammonium oxalate are given in Table I. As was to be expected, little or no change in either the lignin or methoxyl content of the lignin was found. There was a small decrease in the total methoxyl content of the straw owing to removal of pectin amounting to 0.10% and 0.15% for wheat and oat straw respectively. The calculated methoxyl contents of the isolated pectin fractions were 9.52% for wheat straw and 7.14% for oat straw. However, methoxyl determinations on these materials yielded only about 50% of these values, indicating incomplete recovery of the pectin fraction. The small decrease in uronic anhydride in

TABLE I

COMPOSITION OF EXTRACTIVE-FREE STRAWS BEFORE AND AFTER EXTRACTION OF PECTIN

	Wheat straw		Oat straw	
	Before	After	Before	After
Lignin (ash free), %	19.50	19.54	20.05	20.19
Methoxyl in lignin, %	15.70	15.64	15.40	15.50
Total methoxyl, %	3.72	3.62	3.78	3.73
Lignin methoxyl, %	3.06	3.06	3.09	3.13
Lignin methoxyl, % of total methoxyl	82.4	84.6	81.7	83.8
Methoxyl in cellulosic fraction (by difference), %	17.6		18.3	
Uronic acid anhydride, %, (carbon dioxide $\times 4$)	2.99	2.94	3.36	3.32
Pectin, %	1.05		0.70	

the pectin-free straws was due to removal of pectin substances. The data for lignin methoxyl permitted calculation, by difference, of the portion of the total methoxyl of the straw that was present in the cellulosic fraction. Values of 17.6% and 18.3% for wheat and oat straw respectively were in reasonable agreement with a value of 19% reported by Schenck and Kurth for wheat straw holocellulose (21). These values are considerably higher than those reported for woods (10, p. 665).

Composition of the Holocelluloses

The yield and partial composition of the holocelluloses prepared from extracted straw are given in Table II. Summation of holocellulose + lignin

TABLE II

METHOXYL AND URONIC ACID CONTENT OF HOLOCELLULOSES PREPARED FROM PECTIN-FREE STRAWS (DRY BASIS)

Yield of holocellulose, %	Wheat straw	Oat straw
	79.8	76.6
Lignin (ash free), %	1.72	2.10
Methoxyl in lignin, %	10.56	9.56
Lignin methoxyl, % (holocelluloses basis)	0.18	0.20
Total methoxyl, %	0.91	1.14
Holocellulose methoxyl, %	0.73	0.94
Holocellulose methoxyl, % (straw basis)	0.58	0.72
Calculated* holocellulose methoxyl (straw basis), %	0.56	0.60
Uronic acid anhydride, %	3.80	4.32
Uronic acid anhydride, % (straw basis)	3.03	3.31

*Total methoxyl of straw minus lignin methoxyl of straw.

accounted for 99.3% of wheat straw and 96.7% of oat straw. Both wheat and oat holocelluloses contained a small amount of lignin. Methoxyl contents of isolated holocellulose lignins were considerably lower than those of straw

lignin, showing that the chloriting treatment caused considerable demethoxylation. Correction of the total methoxyl in the holocelluloses for the methoxyl in the contaminating lignin yielded values of 0.73% and 0.94% for wheat and oat straws, respectively. When computed on the basis of pectin-free straw, these values become 0.58% and 0.72%, as compared with calculated values of 0.56% and 0.60% for wheat and oat straw respectively. The uronic acid content of wheat and oat holocelluloses on recalculation to the basis of straw (pectin-free) showed that no loss occurred in the delignification of the straw.

Isolation of Hemicellulose Fractions from Holocellulose

The preparation of hemicellulose from wood and fibrous plant material is usually carried out with alkali solutions of various strengths. The same method is applicable to holocellulose although prior removal of lignin, to which the hemicelluloses are presumably attached, may affect the solubilities. Of the many fractionations with alkali that have been proposed the method of Wise (27), which gives quantitative recovery, was regarded as most suitable for the present study. The holocellulose fraction was extracted successively with 5% and 24% potassium hydroxide in an atmosphere of nitrogen. The extracted solutions were removed by filtration directly into an excess of glacial acetic acid, the residue was washed with more potassium hydroxide of the same strength and then with water, and the hemicellulose was finally precipitated in the filtrate by the addition of ethanol. The fractions were recovered by centrifuging and finally dried by solvent exchange from ether. The residue, which is arbitrarily termed " α -cellulose", is similar to that obtained by the usual method of extraction of cellulosic material with 17.5% sodium hydroxide. Hemicellulose fractions were soluble in the acetic acid solutions, which had a pH of approximately 4.0, and were precipitated only after the addition of alcohol. This observation indicates that no hemicellulose fraction "A" (Norris and Preece system of separation) was present in wheat and oat straw. Weihe and Phillips (25) found only negligible amounts of this fraction in their study of wheat hemicelluloses. That hemicelluloses appear to have an optimum pH for precipitation was found by Angell and Norris (4) during an investigation of corn cobs. While the hemicelluloses were precipitated by alcohol from acidified solutions in the present study, it appeared worthwhile to investigate the effect of precipitating the fractions from solutions of different pH values. The effects on yield and methoxyl content of the fractions precipitated from solutions of pH 4.0, 5.0, and 6.0 are shown in Table III. The yields at pH 4.0 and 5.0 were quantitative and at pH 6.0 somewhat low. On the basis of the foregoing observations all precipitations were carried out from solutions having a pH of 4.0. The quantitative yields indicate some source of error as yields of 100% are not to be expected since the acetyl groups, which constitute 2 to 3% of the material, are removed by saponification during alkaline extraction. The high results are probably due to difficulty in making an accurate estimation of the ash in hemicelluloses. This difficulty has been discussed by Wise *et al.* (27). As will be shown in the following section there

TABLE III

EFFECT OF pH ON PRECIPITATION OF HEMICELLULOSE FRACTIONS FROM WHEAT STRAW HOLOCELLULOSE

	Residue			Fraction A			Fraction B			Total		
	pH			pH			pH			pH		
	4.0	5.0	6.0	4.0	5.0	6.0	4.0	5.0	6.0	4.0	5.0	6.0
Yield, %	60.3	59.9	58.0	30.2	29.7	29.0	11.3	10.6	8.3	101.8	100.2	95.3
Methoxyl content, %	0.10	0.08	0.12	1.35	1.33	1.36	0.50	0.56	0.53			

was also a loss of methoxyl amounting to about 0.3% of the holocellulose. The methoxyl content of the various fractions was unaffected by the pH of precipitation.

ANALYSIS OF HEMICELLULOSE FRACTIONS AND RESIDUES

Methoxyl Distribution

The yields and methoxyl contents of the hemicellulose fractions isolated from wheat and oat holocelluloses are given in Table IV. The various fractions account for the wheat holocellulose quantitatively; for oat holocellulose the yields are slightly less. Approximately half of the methoxyl groups of the holocelluloses were recovered in fraction A. The over-all recovery of methoxyl was low for both holocelluloses. This observation led to a closer study of the

TABLE IV

METHOXYL CONTENT OF HEMICELLULOSE AND "α-CELLULOSE" RESIDUES OF WHEAT AND OAT STRAW HOLOCELLULOSES

	Fraction A	Fraction B	Residue	Total
<i>Wheat straw holocellulose (Methoxyl content corrected for lignin, 0.73%)</i>				
Yield, %	30.2	11.3	60.3	101.8
Methoxyl, %	1.35	0.51	0.11	
Methoxyl, % (basis of holocellulose)	0.41	0.06	0.07	0.54
Holocellulose methoxyl accounted for in fractions, %	56.2	8.2	9.6	74.0
<i>Oat straw holocellulose (Methoxyl content corrected for lignin, 0.94%)</i>				
Yield, %	30.8	8.45	57.5	96.75
Methoxyl, %	1.43	0.50	0.30	
Methoxyl, % (basis of holocellulose)	0.44	0.04	0.17	0.65
Holocellulose methoxyl accounted for in fractions, %	46.8	4.3	18.2	69.3

methoxyl analysis as applied to the residue portion and to an investigation into the possible types of methoxyl groups in the fractions.

While the modified Viebock and Schwappach method for methoxyl determination gave satisfactory results with straw, holocellulose, and hemicellulose, those with the α -cellulose residues were more difficult to interpret. A small but measurable methoxyl content was found in both wheat and oat residues. The question whether this amount is real or an artifact is raised by the observation of Reeves that an "apparent methoxyl" of 0.2% to 0.3% can be obtained from sucrose, glucose, cellulose, and starch using the Zeisel procedure (17). (There are many modifications of the original Zeisel method; the Viebock and Schwappach method is one of these.) Repetition of Reeves' experiments in this laboratory gave much lower results on sucrose, wheat starch, and purified cotton cellulose. Sucrose gave 0.01% methoxyl after a 50 min. treatment with hydriodic acid; two additional 50 min. periods yielded a scarcely measurable amount of methoxyl. Starch and cotton yielded about 0.05% methoxyl in a 50 min. treatment; the methoxyl decreased in two further 50 min. periods and became negligible in the fourth and fifth 50-min. treatments. Since the apparent methoxyl contents of the wheat and oat α -cellulose residues were larger than those of purified cotton they are considered to indicate the presence of methoxyl groups. Cooke and Hibberts' trimethylamine method (7) for the isolation of methyl iodide, when applied to the residues, gave a methoxyl value of 0.014% for the wheat holocellulose residue compared with a Zeisel value of 0.114%. Oat holocellulose gave a value of 0.17% by the trimethylamine method as compared with a Zeisel figure of 0.301%. When the trimethylamine methoxyls were used in calculating the balance, the discrepancies between the fractions and the holocelluloses became even greater. Nonetheless it was possible to conclude that the α -cellulose residues contained a small amount of methoxyl. This result is not surprising in view of the fact that α -cellulose and 'true' cellulose of wood and straws are known not to be identical (28). It is interesting to note that the wheat straw α -cellulose prepared from Cross and Bevan cellulose with 17.5% sodium hydroxide contained only 0.07% methoxyl by analysis.

Determination of ester methoxyls by von Fellenberg's method yielded 0.018% and 0.044% for wheat and oat holocellulose respectively. No trace of glycosidic methoxyl was found in either wheat or oat holocellulose by a sensitive method developed by Hoffpauir and Reeves (11). Since the contribution of the ester and glycosidic methoxyls was negligible the conclusion must be reached tentatively that ether methoxyl groups, which are regarded as resistant to alkali, must be partially susceptible to such treatment. In a study of hemicellulosic fractions of aspen holocellulose Thomas was able to account for only 76% of the methoxyl groups although the fractions were quantitatively recovered (24).

Uronic Acid Groups

The determination of uronic acid groups, which occur only in small amounts in cellulosic materials, presents considerable difficulty. It is well

known that pure cellulose, starch, and sugars yield small amounts of carbon dioxide on treatment with 12% hydrochloric acid (5, 15). Anderson states that amounts of carbon dioxide less than 0.3% do not represent the presence of uronic acid (2). Norman (15) and Whistler *et al.* (26) have devised a method of determining uronic acid in the presence of carbohydrates based on the rate of evolution of carbon dioxide. For polyuronides, the rate of evolution is initially high and then falls off progressively as decarboxylation proceeds; for nonuronic carbohydrates the rate is approximately linear with time. However, Nevell (14) has shown that such simple sugars as fructose and sucrose behave as polyuronides.

Uronic acid groups in cotton cellulose of various purities can be fairly readily determined because true cotton cellulose can be used as a control for rate studies of carbon dioxide evolution. However, in straws no such convenient standard exists; the α -cellulose of straws has not been shown to be true cellulose devoid of uronic acid groups. The best that can be done is to use an arbitrary control such as purified cotton cellulose. In the present work the carbon dioxide evolved from purified cotton cellulose under specified experimental conditions was subtracted from the carbon dioxide evolved from the various cellulosic fractions. While this procedure was not ideal it provided the only permissible basis of comparison. The usual conditions of decarboxylation using 12% hydrochloric acid for five hours were compared with those proposed by McCready *et al.* of 19% hydrochloric acid for two hours (13). Using oat holocellulose and purified cotton cellulose the following results were obtained:

Treatment	Yield of carbon dioxide, %		Difference
	Oat holocellulose	Cotton	
12% hydrochloric acid for 5 hr.	1.45	0.28	1.17
19% hydrochloric acid for 2 hr.	1.54	0.39	1.15

The conclusion was reached that the shorter time of decarboxylation was as satisfactory as the longer period. Since Whistler *et al.* (26) have shown that a treatment of five hours with 12% hydrochloric acid was sufficient to determine the uronic acid groups in ramie and flax, the two hour treatment with 19% hydrochloric acid was applied to the cellulosic materials used in the present study. The results of the uronic acid analysis of the hemicellulosic fractions and α -cellulose residues are given in Table V. Almost quantitative recovery of the uronic acid groups was achieved in the various fractions of wheat and oat holocelluloses. About 70% of the uronic acid was contained in Fraction A. This observation supports the general claim that the polyuronides constitute the readily soluble fraction of holocellulose. The small amount of uronic acid in the α -cellulose residues showed that they are not identical with a true cellulose which is composed only of glucose residues.

TABLE V

URONIC ACID ANHYDRIDE CONTENT OF HEMICELLULOSE AND " α -CELLULOSE" RESIDUE OF WHEAT AND OAT HOLOCELLULOSES

	Fraction A	Fraction B	Residue	Total
<i>Wheat straw holocellulose (Uronic acid anhydride content 3.80%)</i>				
Yield, %	30.2	11.3	60.3	101.8
Uronic acid anhydride, %	8.68	5.36	0.80	
Uronic acid anhydride, % (basis of holocellulose)	2.62	0.60	0.48	3.70
Holocellulose uronic acid accounted for in fractions, %	69.0	15.8	12.6	97.4
<i>Oat straw holocellulose (Uronic acid anhydride content 4.32%)</i>				
Yield, %	30.8	8.45	57.5	96.75
Uronic acid anhydride, %	10.20	6.40	0.76	
Uronic acid anhydride, % (basis of holocellulose)	3.14	0.54	0.44	4.12
Holocellulose uronic acid accounted for in fractions, %	72.7	12.5	10.2	95.4

Molar Ratio of Uronic Acid Anhydride to Methoxyl Groups

The molar ratio of uronic acid anhydride to methoxyl groups in holocellulose, hemicellulose A and B, and the α -cellulose residues of wheat and oat holocelluloses are given in Table VI. For the holocelluloses, the molar ratios were

TABLE VI
MOLAR RATIO $\frac{\text{URONIC ACID ANHYDRIDE}}{\text{METHOXYL}}$ IN WHEAT AND OAT
HOLOCELLULOSES AND THEIR HEMICELLULOSE FRACTIONS

	Wheat	Oat
Holocellulose	0.92	0.82
Fraction "A"	1.33	1.07
Fraction "B"	2.20	1.54
" α -Cellulose" residue	1.10	0.47

less than unity. A value of 1.29 can be calculated from data published by Schenck and Kurth for wheat straw holocellulose (21); a similar calculation applied to data reported by Thomas for aspen holocellulose yields a molar ratio of 1.07 (24). The proximity of these values to 1 suggests the presence of hexuronic acid groups containing one methoxyl. In fractions A and B the molar ratio was greater than unity. The relatively high ratio in fraction B indicates that some uronic acid residues may not contain a methoxyl group. The uncertainty of the analytical methods, especially for methoxyl as applied

to the residues, makes calculations of the ratios of doubtful value. The possibility cannot be overlooked that some of the uronic acid in the residues may have been formed by oxidation during the fractionation procedures. A hemicellulose fraction extracted from white birch by ethylenediamine has been reported by Sith (22) to contain a $\frac{\text{uronic acid}}{\text{methoxyl}}$ molar ratio of 1.6. Various hemicellulosic fractions isolated by Sands and Nutter (20) from mesquite wood contained a $\frac{\text{methoxyl}}{\text{uronic}}$ ratio of 1.0. While most investigators have presumed that the methoxyl group is attached to the uronic acid residue, a report by Anderson and Kinsman on the isolation and identification of an aldobionic acid from cottonseed hulls does not mention methoxyl groups being found in the isolated glucuronic acid portion (3).

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SEPARATION OF P^{32} FROM NEUTRON-IRRADIATED SULPHUR¹

BY A. H. BOOTH

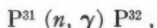
Abstract

A method for extracting P^{32} from neutron-irradiated sulphur has been developed. The sulphur is melted under a mixture of acetic acid and acetic anhydride at boiling temperature, and the P^{32} is recovered from the residue left after distilling the acid. An apparatus for carrying out the process by remote control is described. It has been used for production of 100 millicurie amounts of carrier-free P^{32} at the Chalk River laboratories.

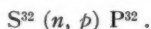
Introduction

P^{32} is one of the more commonly used radioisotopes in tracer work, and preparations having high activity and high specific activity are frequently required. The method described below has been used for separation and purification of 100 millicurie amounts.

In the nuclear reactor or "pile", P^{32} can be synthesized by two methods. The direct irradiation of phosphorus with slow neutrons,

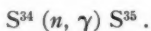


gives a high yield, but the specific activity attainable by the method is limited. A much greater specific activity is given by irradiation of sulphur with fast neutrons,



The P^{32} can then be separated chemically from the parent sulphur.

Elemental sulphur is the most suitable target material. It is thermally stable and easily obtained free from objectionable impurities. Undesirable side reactions are thus minimized; the only other important radioactive product is S^{35} formed by the reaction,



The phosphorus atoms, when formed, are locked inside the sulphur crystal lattice, and no separation can be made until the crystal structure is broken by melting or solution. Because of its minute concentration the phosphorus may then react very quickly with trace impurities, particularly oxygen. Until complete oxidation has been ensured the possible coexistence of several valence states must be considered.

Separation can be made by several methods, but when highly active material is processed the simplicity of the method chosen is of prime importance. Manipulations must be carried out from behind a shield sufficiently thick to protect the operator from the high energy beta rays. Consequently, each

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operation must be adaptable to remote control. A high yield is secondary to this consideration.

Preliminary experiments by others (2) in this laboratory indicated that good separation could be made either by dissolving the sulphur in carbon disulphide and extracting the P^{32} with dilute nitric acid, or by leaching the molten sulphur with superheated water in a pressure vessel. These methods presented some difficulties and hazards in remote control operation. Cohn (1) has described a method for extracting P^{32} with concentrated nitric acid. The constant boiling temperature of the acid is just high enough to keep the sulphur molten during extraction. This procedure also is difficult to adapt to remote control because the sulphur must be melted separately and poured slowly into the boiling acid. Furthermore a considerable amount of sulphuric acid is formed, and the S^{35} introduced into the solution can cause some difficulty in subsequent steps.

Experimental

Extraction of P^{32} by Organic Liquids

Extraction with a liquid at ordinary pressure is one of the less difficult operations to adapt to remote control. For this reason a number of organic liquids with boiling points in the range 120° to 150° C. were examined as possible extracting agents. The case of acetic acid is somewhat exceptional in that its boiling point, 118° C., is slightly less than the melting temperature of sulphur. There is ordinarily enough superheat at the wall of the flask to overcome this, and the melted sulphur then can be dispersed readily because it does not resolidify until the temperature falls below 112° C. Occasional failures were noted, and it was found necessary to raise the boiling point somewhat by adding acetic anhydride.

Yield tests were carried out as follows. Neutron-irradiated sulphur was heated with the solvent under reflux until melted. The solvent was boiled for 15 min. while the sulphur was agitated with a current of air. After cooling, the solvent was decanted, and its P^{32} content determined using a conventional type of Geiger counter (3). A 25 mgm. per cm^2 aluminum absorber was placed between sample and counter tube to eliminate any radiation due to S^{35} activity. The sulphur residue was oxidized to sulphuric acid with a mixture of nitric acid and bromine and then tested in the same way.

Some results are shown in Table I. Acetic acid gave a much higher yield than the nonpolar solvents. With successive extractions a maximum value was soon reached, the nonextractable portion presumably being combined with impurities such as iron. The greater efficiency of the acid solvents may be explained by a tendency to dissociate such compounds. Excessive iron or aluminum impurity lowers the yield, but variation of iron content in the range 25 to 100 p.p.m. has no appreciable effect. The values given refer to a technical grade roll sulphur containing about 25 p.p.m. of iron.

The mechanism of the extraction was not determined but it is unlikely that elemental phosphorus is involved. More probably the phosphorus reacts

TABLE I

YIELDS OF P^{32} EXTRACTED FROM NEUTRON-IRRADIATED SULPHUR BY ORGANIC LIQUIDS

Extracting liquid	Vol., ml./gm. of sulphur	P^{32} extracted, %		
		One extraction	Two extractions	Three extractions
Glacial acetic acid	5	83	90	92
Acetic acid - acetic anhydride mixture 3 : 1 by vol.	2	70	85	88
	5	80	87	89
	10	81	88	90
Propionic acid	5	57	69	—
Isoamyl acetate	5	56	58	—
Isoamyl alcohol	5	58	59	—
Diisopropyl ketone	5	55	57	—

first with traces of oxygen and water and is taken into solution as phosphorous and phosphoric acids. Evidence to support this view is the fact that nearly all the P^{32} remains in the nonvolatile residue when the sulphur is sublimed in air.

Distillation, Recovery, and Purification

Acetic acid and the acetic acid - acetic anhydride mixture were selected for further study. When the extract is decanted from the sulphur and distilled, all the P^{32} remains in the residue. This residue is chiefly sulphur carried over in solution. The solubility of sulphur in glacial acetic acid is about 1 gm. per 100 ml. at boiling, and about 0.05 gm. per 100 ml. at room temperature. On cooling, the dissolved sulphur separates in large crystals from which the solution can easily be decanted. The distillation is carried out at a low temperature in the final stages to prevent the formation of plastic sulphur.

The P^{32} can be leached from the residue by boiling with dilute mineral acid. The percentage recovered depends on the amount of residue and conditions of distillation, but is always high, provided the residue has not been overheated. Under the conditions described later recoveries of 90% to 96% were obtained.

In the resulting solution the P^{32} is present as orthophosphate ion only. Meta and pyrophosphates formed in the residue would be hydrolyzed to orthophosphate during the boiling leach. Phosphites were shown to be absent by adding sodium phosphite carrier and reducing with zinc in hydrochloric acid. The evolved phosphine contained negligible P^{32} activity.

At this point the solution contains no sulphur or sulphuric acid, but trace impurities such as iron and organic matter are sometimes present. The purification procedure described by Cohn (1) gave good results. The phosphate is precipitated by ammonia on ferric or lanthanum hydroxide carrier. A basic phosphate is formed which coprecipitates with the hydroxide. Sodium or potassium hydroxide cannot be used as precipitant because the basic

phosphate is then not formed. Subsequently, cations are removed by dissolving the hydroxide in hydrochloric acid and passing the solution through a base-exchange resin. The resin used in this work was Dowex-50. It was conditioned before use by three or four sodium-hydrogen exchange cycles, ending finally in the hydrogen form.

Remote Control Apparatus

The procedure outlined above is readily adapted to remote control. The apparatus used in some experimental runs is shown in Fig. 1. It is enclosed behind a $\frac{3}{8}$ in. screen of "Lucite" sheet, and operated entirely by remote control. Since no gamma rays are emitted this shield provides adequate

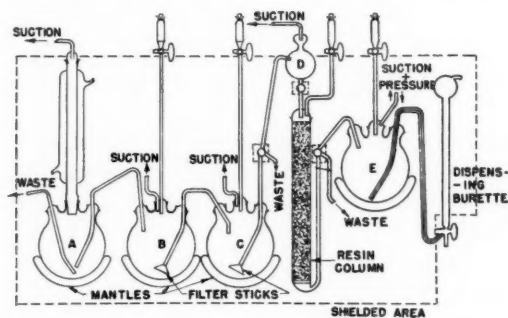


FIG. 1. Remote control apparatus for P^{32} extraction.

protection. The three stopcocks indicated by the dotted squares are controlled by rods extending through the shield, but all other valves are accessible directly. Suction and pressure lines are brought together on stopcock manifolds mounted on a control panel.

From 20 to 100 gm. of sulphur is introduced into *A* through the condenser and a 3 : 1 mixture of acetic acid and acetic anhydride added in an amount corresponding to 2 to 5 ml. per gm. of sulphur. The condenser cap is replaced and the mixture heated. When the sulphur has melted under the boiling liquid it is agitated for about 30 min. by a stream of air drawn through the siphon tubes. The air stream is maintained during cooling so that the ends of the siphon tubes do not become sealed.

Two extractions are made and the extracts combined in *B*, then distilled back into *A*, using slight vacuum in the receiver. About 100 ml. of 0.1 *N* hydrochloric acid is run in to *B*, boiled for five minutes and drawn over into *C* through the filter stick.

The hydroxide precipitation is carried out in *C*. Four milligrams of ferric chloride is added and precipitated with 20 to 25 ml. of 1.0 *N* ammonia. The solution is boiled, cooled somewhat, and the supernatant drawn over into a waste flask through the filter stick. The precipitate is washed with water, then redissolved in 100 ml. of hot 0.05 *N* hydrochloric acid, drawn up

into *D*, and passed through the resin column. The outlet tube of the column is bent up into a position only a few inches below the top of the column packing, so that the column cannot run dry inadvertently.

The effluent is dispensed directly as carrier-free P^{32} in acid solution, or it is evaporated to dryness in *D* and taken up in neutral solution. In the second case addition of a small amount of inactive phosphate is necessary to prevent adsorption on the walls of the container.

The waste sulphur is remelted under acetic anhydride and drawn over quickly into a disposable flask outside the shield. Liquid wastes in holding flasks (not shown) are run to drain, the siphon being started with a pressure bulb. Flask *B* is cleaned periodically with concentrated sulphuric acid.

Discussion

An apparatus similar to that described has been operated for some months at Chalk River. The arrangement is compact and the entire procedure can be carried out in a few hours. Activities greater than 100 millicuries have been separated without difficulty.

Modifications in technique are still being made and under the changing conditions over-all yields have varied from 50 to 80%. Some loss occurs in each stage, because, at this minute concentration of phosphate, it is difficult to prevent entirely an irregular adsorption on the apparatus and column filling. If a few milligrams of inactive phosphate are added early in the process, losses are minimized, but this, of course, lowers the specific activity of the product. In general, yields decrease with increasing size of batch treated. This can probably be accounted for by less efficient agitation, larger distillation residue, and greater total quantity of impurities introduced. The method in its present form is limited, therefore, to amounts of sulphur less than 150 gm. The availability at Chalk River of high neutron fluxes makes targets of this size entirely adequate.

Acknowledgments

The author wishes to express his thanks to Mr. G. P. Maxwell, who carried out some of the high activity runs, and to Mr. N. L. F. Jupe for assistance in the experimental work.

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1,10-PHENANTHROLINE, 2,2'-BIPYRIDYL, AND THE PLATINUM METALS¹

BY D. E. RYAN²

Abstract

A previously described method for the determination of palladium with 1,10-phenanthroline is herein further extended and investigated. In all cases the complex is used as the weighing form for palladium, the theoretical factor 0.2982 being used to calculate the palladium content. The reactions of palladium, platinum, iridium, rhodium, and gold with 1,10-phenanthroline and 2,2'-bipyridyl are described. Palladium may be separated from rhodium by a single precipitation, using 1,10-phenanthroline as precipitant. A double precipitation enables palladium to be separated from platinum and iridium.

Introduction

The use of compounds containing the active functional group $\text{=N}-\overset{\parallel}{\text{C}}-\overset{\parallel}{\text{C}}-\text{N=}$, to which 2,2'-bipyridyl and 1,10-phenanthroline owe their capacity to react, as precipitants for certain cations and anions has not been studied extensively. DeVries (1) reported the separation and microdetermination of ferrous iron and determined perchlorates, periodates, and persulphates in the presence of chlorate, iodate, and sulphate, using 5-nitro-1,10-phenanthroline ferrous sulphate as precipitant. Gillis (2) studied the reactions of ferroin with different anions in water and in hydrofluoric acid medium, and Ryan and Fainer (3) used 1,10-phenanthroline to precipitate palladium from palladous chloride solutions. The following report is the result of further investigation into the use of these reagents for platinum metal determination.

Experimental and Results

Reagents

1. 1,10-Phenanthroline: 0.5 gm. of 1,10-phenanthroline dissolved in 100 ml. of water.
2. 2,2'-Bipyridyl: 0.5 gm. of 2,2'-bipyridyl dissolved in 100 ml. of water.
3. Palladous chloride solutions—prepared by dissolving $\text{PdCl}_2 \cdot 2\text{H}_2\text{O}$ in 0.5 *M* hydrochloric acid solution and standardized by the dimethylglyoxime method.
4. Palladous nitrate, palladous sulphate, platinic chloride, rhodium chloride, iridium tetrachloride, and gold chloride solutions—prepared from the metal or suitable pure salt and containing approximately 1 mgm. of the metal per ml. of solution.

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Reactions of Palladous Salts with 1,10-Phenanthroline and 2,2'-Bipyridyl

1,10-Phenanthroline and 2,2'-bipyridyl react with palladous salts to give complexes of the type $\text{PdC}_{12}\text{H}_8\text{N}_2\text{Cl}_2$. The complex formed with 1,10-phenanthroline is a pale yellow solid, while that with 2,2'-bipyridyl is a brighter yellow. The 1,10-phenanthroline complex is very insoluble, while that with 2,2'-bipyridyl is slightly soluble in water. Palladous nitrate gives with 1,10-phenanthroline a fine crystalline yellow precipitate that has a similar composition, $\text{PdC}_{12}\text{H}_8\text{N}_2(\text{NO}_3)_2$. The dry compound is slightly soluble in cold but appreciably soluble in hot water. Palladous sulphate gives no precipitate on the addition of 1,10-phenanthroline, but a flocculent precipitate forms immediately on the addition of chloride to this solution. On the addition of halides other than chloride to a solution of 1,10-phenanthroline and palladous sulphate, the following results are obtained: fluoride gives a white gelatinous precipitate, bromide gives a yellow flocculent precipitate, and iodide gives a heavy brown precipitate. The composition of these precipitates has not been investigated.

Reactions of Platinum, Rhodium, Iridium, and Gold with 1,10-Phenanthroline and 2,2'-Bipyridyl

In a previous paper (3) it was reported that no precipitates were obtained with platinum, rhodium, and iridium, using 1,10-phenanthroline. The conditions used in obtaining these results involved dilute solutions (0.05 mgm. of Pt metal per ml. of solution) that were maintained near the boiling point for two hours. Further investigation shows that, in more concentrated solutions, precipitates are obtained from platinum (PtCl_6^-) and iridium (IrCl_6^-) solutions. The platinum precipitate is orange yellow, while the iridium precipitate is reddish brown. Precipitation is incomplete, with little precipitate being obtained in dilute solutions. Rhodium gives no precipitate even from concentrated solution. 2,2'-Bipyridyl reacts similarly to 1,10-phenanthroline.

Gold is precipitated, as a complex, from hydrochloric acid solutions. This canary yellow precipitate dissolves on heating. Since no yellow color was noted in the filtrate from the precipitation of gold (in the cold), a series of tests was carried out to determine if the precipitation is complete. Using 2,2'-bipyridyl as precipitant, incomplete precipitation was found in all cases where hydrochloric acid concentration was varied from 1 to 20%. Similar results were obtained using 1,10-phenanthroline. The stannous chloride spot test was used to test for completeness of precipitation.

The Determination of Palladium with 1,10-Phenanthroline and 2,2'-Bipyridyl

The procedure for the determination of palladium with 1,10-phenanthroline was essentially that previously described (3). Since, however, the composition of the complex is constant and has a low palladium content, all results unless otherwise indicated were obtained using $\text{PdC}_{12}\text{H}_8\text{N}_2\text{Cl}_2$ as the weighing form for palladium. Samples of the standard palladous chloride solution were measured into 250 ml. beakers, the volume made up to approximately 150 ml.,

and the palladium precipitated with 1,10-phenanthroline. These solutions were allowed to stand for two hours, and the precipitates were filtered through medium porous porcelain crucibles, washed with 1% hydrochloric acid, dried at 110° C., and weighed as the complex. The results obtained by this procedure are shown in Table I. The quantity of palladium was calculated, using the theoretical factor 0.2982.

TABLE I
DETERMINATION OF PALLADIUM WITH 1,10-PHENANTHROLINE,
WEIGHING AS $\text{PdC}_{12}\text{H}_8\text{N}_2\text{Cl}_2$

No.	Palladium taken, mgm.	Palladium recovered, mgm.
1	11.04	11.05
2	11.04	11.01
3	11.04	11.03
4	11.04	11.03
5	11.04	11.06
6	11.04	11.03
7	11.04	11.00
8	11.04	11.03
9	10.86	10.86
10	10.86	10.89
11	10.86	10.86

The results obtained by a similar procedure with 2,2'-bipyridyl are shown in Table II. These results were obtained using $\text{PdC}_{10}\text{H}_8\text{N}_2\text{Cl}_2$ as the weighing form and the theoretical factor 0.3124.

TABLE II
DETERMINATION OF PALLADIUM WITH 2,2'-BIPYRIDYL

No.	Palladium taken, mgm.	Palladium recovered, mgm.	Volume solution, ml.
12	10.86	10.88	50
13	10.86	10.86	75
14	10.86	10.84	100
15	10.86	10.48	200
16	10.86	10.10	300
17	10.86	9.01	400

Table II shows the effect of concentration on the degree of precipitation, using 2,2'-bipyridyl as precipitant. Similar experiments with 1,10-phenanthroline showed no such deviations, complete precipitation being obtained in all cases. When 10.86 mgm. of palladium was taken the palladium recovered from 400 ml. of solution, using 1,10-phenanthroline, weighed 10.86 mgm. A second sample weighed 10.87 mgm.

The Precipitation of Palladium from Solutions Containing Other Platinum Metals

Since dilute solutions of platinum metals other than palladium gave only slight precipitates with 1,10-phenanthroline, attempts were made to separate palladium from platinum, iridium, and rhodium.

The palladium was precipitated as previously described from solutions "salted" with varying amounts of the other metals. Results 18 to 21 in Table III show that palladium may be separated from rhodium by a single precipitation. Results 22 to 25, obtained in the presence of iridium and platinum, are approximately 2% high.

TABLE III

DETERMINATION OF PALLADIUM IN PRESENCE OF OTHER PLATINUM METALS

No.	Palladium taken, mgm.	Palladium recovered, mgm.	Platinum metal added, mgm.
18	11.04	11.01	5 Rh
19	11.04	11.00	5 Rh
20	11.04	11.00	5 Rh
21	11.04	11.03	10 Rh
22	11.04	11.10	3 Ir
23	11.04	11.16	6 Ir
24	10.86	11.02	6 Pt
25	10.86	11.05	6 Pt
26	10.84	10.81	10 Pt
27	10.84	10.83	10 Pt
28	10.84	10.78	10 Pt
29	4.77	4.72	20 Pt
30	4.77	4.73	6 Ir
31	4.77	4.72	6 Ir

Reprecipitation Method

Since high results were obtained by a single precipitation when platinum and iridium were present, a double precipitation was attempted. The precipitate was filtered through retentive filter paper, and nitric and sulphuric acids were used to destroy organic matter. The solution was evaporated to sulphuric acid fumes, diluted with 1% hydrochloric acid after filtration and the palladium reprecipitated. The precipitate was very yellow and the results obtained, the precipitate being weighed as $\text{PdC}_{12}\text{H}_8\text{N}_2\text{Cl}_2$, were 5% high. It was subsequently discovered that the procedure using nitric and sulphuric acids resulted in nitration of the 1,10-phenanthroline, with incomplete destruction of the organic matter.

Although satisfactory results should be obtained on ignition of the above precipitate and weighing as the metal, an alternative procedure was used that allows use of $\text{PdC}_{12}\text{H}_8\text{N}_2\text{Cl}_2$ as the weighing form. The samples, after the first precipitation, were filtered through ashless filter paper and ignited over a burner. These residues were transferred to conical flasks, which were

closed with short-stemmed funnels, and dissolved in aqua regia. The solutions were evaporated several times in the presence of excess hydrochloric acid, filtered, diluted, and the precipitation carried out as previously described. Results 26-31 in Table III show that palladium may be separated from platinum and iridium by this method. Attempts to separate palladium from gold by the same procedure were unsuccessful.

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THE THERMAL DECOMPOSITION OF CUMENE HYDROPEROXIDE IN RELATION TO CERTAIN ASPECTS OF EMULSION COPOLYMERIZATION¹

By J. W. L. FORDHAM AND H. LEVERNE WILLIAMS

Abstract

Cumene hydroperoxide decomposes near 100° C. in reactive solvents but only slightly in stable solvents. The decomposition is not a simple first order reaction. The results may be interpreted as the sum of a first and a three-halves order reaction. Approximate over-all energies of activation of the decomposition determined from the data obtained for the decomposition in cumene (31 kcal. per mole), methanol (31 kcal. per mole), styrene (20 kcal. per mole), and the energy of activation for the initial unimolecular step in benzene-styrene mixtures (30.4 kcal. per mole) agree with the values reported for similar peroxides and hydroperoxides in similar solvents. The energy of activation for the radical-induced decomposition is 6.5 kcal. per mole. The data suggest that the first step of the decomposition is the unimolecular reaction involving scission of the O-O bond. If the resultant $C_6H_5C(CH_3)_2O^{\cdot}$ radical is not reactive towards the solvent, it tends to lose either a phenyl or a methyl radical and form acetone or acetophenone respectively. The former reaction predominates. The decomposition rapidly becomes autocatalytic, particularly in cumene at higher temperatures, presumably because of the radical-induced decomposition of the hydroperoxide. The identified products of decomposition in cumene can be explained by the various reactions of the radicals formed in the mixture. The polymerization shortstop 2,5-ditertiarybutylhydroquinone reacts rapidly below 0° C. with an equimolar weight of cumene hydroperoxide, and the resulting products retard further thermal decomposition at 90° C. quite strongly. The shortstop 2,4-dinitrochlorobenzene does not react with cumene hydroperoxide and would seem to act merely as a powerful retarder for the decomposition at the higher temperature. The effect of other related compounds on the decomposition of cumene hydroperoxide in styrene was less marked and agrees with the relatively less effectiveness of these compounds as stoppers in emulsion copolymerization.

Introduction

Organic peroxides and hydroperoxides are initiators of free-radical-induced polymerization reactions. Hence a knowledge of the mechanism of their decomposition is becoming important in this field. Hydroperoxides are present as intermediate compounds in the oxidation of hydrocarbons, including hydrocarbon polymers. Their decomposition plays a role in the degradation of these polymers and in the drying of oils. Considerable research has been carried out on the decomposition of peroxides, but as yet there is very little pertinent data available on hydroperoxides such as cumene hydroperoxide, which is used currently as an initiator in the low temperature butadiene-styrene copolymerization recipes (17, 21). Consequently a preliminary study of the decomposition of this compound as related to polymerization initiation and stopping was undertaken. The results obtained in the thermal decomposition are presented in this report. No similar published data for this compound have been found.

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The literature revealed that certain types of reactions are common to the decomposition of peroxides and hydroperoxides in solvents. The initial scission is accepted to be at the central O-O bond. If the radical that is formed contains the -COO- group there is a marked tendency for carbon dioxide to be split off, leaving a new radical (7, 13), particularly when the initial radical does not react readily with the solvent molecule (13) or when the temperature is increased (14). However, if the initial radical contains the -CO- group, there is a tendency for C-C fission to take place at the weakest adjacent bond of the α -carbon atoms to yield a ketone or aldehyde (9, 20, 26, 27) especially when the temperature is increased (20). This reaction is thermodynamically possible because the energy required to split the C-C bond is balanced by that released in the formation of the C=O bond from the C-O bond. Both the initial radical and the resultant radical produced by it can abstract an α -hydrogen atom from the solvent if this atom is available (5, 6, 13, 14, 15, 20). These radicals attack olefin molecules at the α -carbon atoms and at the double bond (7), thereby initiating polymerization. Then to stop polymerization at the desired degree of conversion of hydrocarbons to rubber some agent must be added to inactivate the free radicals present and prevent the formation of further free radicals.

It would be desirable but very difficult to study the decomposition of cumene hydroperoxide under conditions present during emulsion copolymerization. However, the mechanism of emulsion polymerization is considered to be the same as of bulk polymerization (8). Hence it is a great simplification to study those reactions known to take place in the oil phase by comparatively simple solution techniques and apply the results to the emulsion system, allowance being made for the influence of aqueous phase. For this reason the thermal decomposition of cumene hydroperoxide was studied in hydrocarbon solutions. Since all the hydrocarbons are slightly soluble in water and are solubilized and dispersed by the emulsifying agents, some refinement of the results will be required in applying them to the emulsion system.

Methods

Analysis for Cumene Hydroperoxide

Of the methods of analysis for peroxides and hydroperoxides disclosed in the literature (1, 18, 22, 23, 24) the iodometric method, which yields precise results from a simple procedure, seemed the most desirable to adopt for cumene hydroperoxide. Nozaki's modification of this method was tested first. The results depended upon the reaction time between sodium iodide and cumene hydroperoxide in acetic anhydride medium both at room temperature and at a higher temperature obtained by placing the flask on a hot plate set at low heat. This method also has the disadvantage that individual portions of sodium iodide must be weighed for each analysis. A stock solution of sodium iodide in acetic anhydride cannot be made because the iodide is oxidized continually to iodine by the atmospheric oxygen, the supply of which is renewed every time that the solution is exposed to the atmosphere.

Because of these disadvantages the method suggested by the Hercules Powder Company* was tested. In this method the sample is added to 100 ml. of 99% isopropanol in a glass-stoppered Erlenmeyer. Ten milliliters of glacial acetic acid and 1 ml. of saturated potassium iodide solution are added and the mixture is refluxed gently (to avoid the loss of iodine) for three to four minutes. The condenser is washed with 10 ml. of isopropanol, 25 ml. of water is added, and the flask stoppered. When the resulting solution has cooled somewhat, the liberated iodine is titrated with standard thiosulphate solution to the disappearance of the yellow color. Starch cannot be used as an indicator, but this is not a serious disadvantage unless the solution is objectionably colored. A blank is measured on the reagents. This procedure is somewhat simpler than the previous one and gives reproducible results that are independent of the time of refluxing up to 10 min. and gives only very small errors up to 25 min. refluxing. It was the method chosen for the subsequent investigation.

Purification of the Cumene Hydroperoxide

Commercial cumene hydroperoxide supplied by the Hercules Powder Company assayed approximately 70% active material and was purified by the method used by that Company*. One hundred milliliters of the crude hydroperoxide was slowly added to 300 ml. of a 25% sodium hydroxide solution in water. The mixture was agitated well and cooled to maintain the temperature below 30° C. Crystals of the sodium salt were formed and were filtered from the caustic solution. The filtered crystals were washed twice with 25 ml. portions of benzene and then stirred in 100 ml. of benzene for 20 min. The sodium salt was filtered and the washing operation repeated, using 100 ml. of fresh benzene. The sodium salt was filtered once more, washed with two more 25 ml. portions of benzene, and suspended in 100 ml. of distilled water. The pH was lowered to 7.5 with 4 *N* hydrochloric acid, the hydroperoxide extracted twice, using 20 ml. portions of *n*-pentane. The moisture and solvent were removed under vacuum at room temperature and the last traces were removed at 1 mm. pressure and 40° to 50° C. The recovery was 85% to 90% of a material that ranged in purity from 97% to 100% active hydroperoxide.

Decomposition of Cumene Hydroperoxide in Solvents

The solvent was subjected to a distillation at atmospheric pressure (except styrene, which was distilled at 1 mm. pressure to prevent polymerization) in order to remove any dissolved oxygen from the solvents, and any inhibitor from the styrene and the middle third was collected. Unless otherwise stated a 2% by weight solution of 98% cumene hydroperoxide was made, using the freshly distilled solvent. A definite volume of the solution was transferred to each reaction tube by means of a pipette. The reaction tubes consisted of 12-in. lengths of 9 mm. glass tubing that had been rinsed previously with distilled water, dried, and sealed off at one end. The tubes containing

* Private communication.

Source of Chemicals

Solvent	Source	Grade	Purification	$\eta_{\frac{25}{D}}$
Cumene	Polymer Corp. Ltd.	Mixture of alkylated benzenes	Fractionated at atmospheric pressure in a 65 plate Stedman column at a 10:1 reflux ratio	1.4887
Methanol	E. I. du Pont de Nemours	Synthetic	Same. Dried over potassium carbonate	1.3275
Styrene	Polymer Corp. Ltd.	Technical	None	1.5438
Benzene	Algoma Steel Co.	Technical	Fractionated at atmospheric pressure in a 65 plate Stedman column at a 10:1 reflux ratio	1.4980
<i>n</i> -Hexane	Phillips Pet. Co.	Technical	None	1.3729
2,5-Ditertiarybutyl-hydroquinone	Eastman Kodak Co.	Practical	Recrystallized from benzene	—
2,5-Ditertiarybutyl-hydroquinone	Monsanto	Recrystallized	None	—
2,4-Dinitrochlorobenzene	Eastman Kodak Co.	Pure	Recrystallized from benzene	—
2,6-Ditertiarybutyl-4-methylphenol	Standard Oil Co.	Deenax	Recrystallized from benzene	—
Phenyl- β -naphthylamine	Canadian Industries Ltd.	Neozene D	Recrystallized three times from methanol	—

the peroxide solution were immersed in a dry ice-ethanol bath. In groups of four these were evacuated, flushed with commercial nitrogen, evacuated, flushed, evacuated, and sealed off under vacuum in succession, so that they contained approximately the same vapor volume above the solution and were approximately 10 in. in length. The tubes were transferred from the dry ice-ethanol bath to a constant temperature ethylene glycol bath maintained within one-tenth of a centigrade degree. Each tube was left in the bath for the desired reaction time and then quenched in the dry ice-ethanol bath. The tube was opened and the residual hydroperoxide was determined iodometrically, using the procedure of the Hercules Powder Company as outlined previously, with the modification that 10 ml. of the isopropanol was held back from the titration flask in order to rinse the reaction tube. In the later studies the reaction tube was rinsed with 10 ml. of toluene before the alcohol to ensure complete removal of the sample. The undecomposed sample for every series of results was analyzed to check the strength of the original solution, and this was taken as the initial concentration.

Results

Decomposition of Cumene Hydroperoxide in Single Solvents

The results for the thermal decomposition at 90.5°, 99.7°, and 109.4° C. of a 2% solution of cumene hydroperoxide in cumene, methanol, and styrene as representative solvents are given in Figs. 1 to 3 respectively. The accuracy

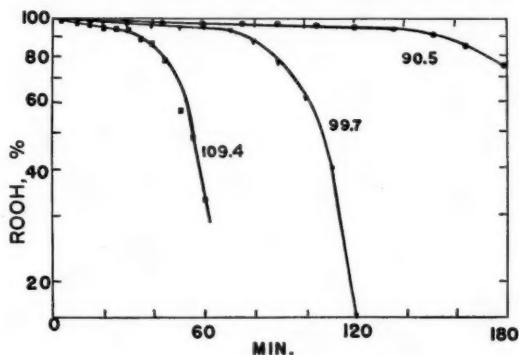


FIG. 1. Per cent residual cumene hydroperoxide versus time at 90.5, 99.7, and 109.4° C. in cumene.

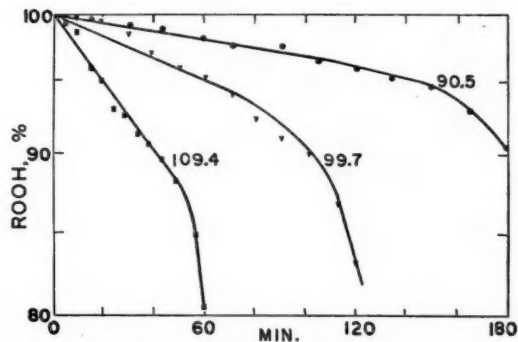


FIG. 2. Per cent residual cumene hydroperoxide versus time at 90.5, 99.7, and 109.4° C. in methanol.

of the results obtained after considerable polystyrene had formed was decreased because the high viscosity of the solution made it difficult to remove the entire sample. The graph of the logarithm of the unreacted cumene hydroperoxide against time shows that the peroxide disappears initially much more rapidly in styrene than in either cumene or methanol in which the rates are approximately equal. The decomposition appears to be autocatalytic in all cases and this effect is much more pronounced in cumene. In the latter case the final rate of disappearance is the fastest of all those measured. The autocatalytic effect is greater at the higher temperatures.

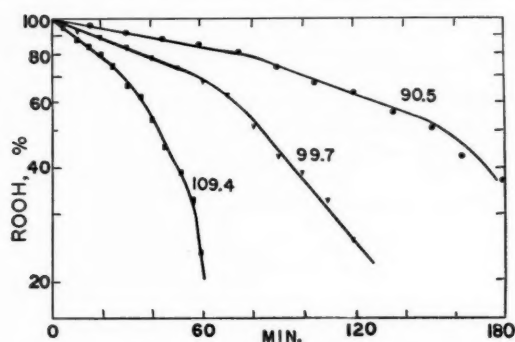


FIG. 3. Per cent residual cumene hydroperoxide versus time at 90.5, 99.7, and 109.4° C. in styrene.

The decomposition is not first order but if the graphs are assumed linear during the first few per cent decomposition then rate constants can be calculated from large scale graphs. The data are in Table I. Approximate

TABLE I
EFFECT OF TEMPERATURE ON RATE CONSTANTS IN VARIOUS SOLVENTS

T° A	Cumene	Methanol	Styrene
363.7	$2.1 \times 10^{-4} \text{ min.}^{-1}$	$2.9 \times 10^{-4} \text{ min.}^{-1}$	$3.05 \times 10^{-3} \text{ min.}^{-1}$
373.9	6.0	8.2	6.25
382.6	18	24	11.90

activation energies calculated from these data yield a value of 31 kcal. per mol. for the decomposition in cumene or methanol and 20 kcal. per mol. in styrene as solvent. These values are in good agreement with those reported for similar peroxides and hydroperoxides in similar solvents (3, 10, 16, 25).

Repetition of the work at 90.5° C., using two initial concentrations of cumene hydroperoxide (1% and 2%), yielded the data in Fig. 4. Calculation of the first order rate constants for disappearance gave the results in Table II. The variation of the constants with concentration even over this narrow range suggest further that the reaction is not first order.

TABLE II
EFFECT OF CONCENTRATION ON RATE CONSTANT IN VARIOUS SOLVENTS

Solvent	Cumene 2% hydroperoxide	Cumene 1% hydroperoxide
Cumene	$2.2 \times 10^{-4} \text{ min.}^{-1}$	$2.1 \times 10^{-4} \text{ min.}^{-1}$
Methanol	1.5	1.2
Styrene	30.5	26.5

Comparison of the data in Tables I and II and Figs. 2 and 4 will show that in Table II and Fig. 4 the rate of disappearance in methanol has diminished with the use of a new distillation batch from the same can of the solvent. This

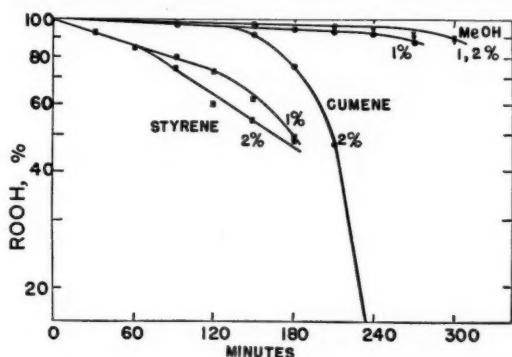


FIG. 4. Per cent residual cumene hydroperoxide versus time at 90.5° C. for two initial concentrations of hydroperoxide.

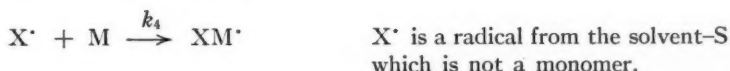
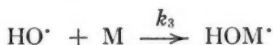
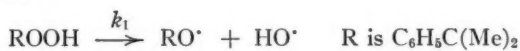
emphasizes the need for uniform batches of materials in these studies. It will be noted too that the autocatalytic effect in cumene is not as evident with the lower concentration of cumene hydroperoxide. Otherwise the agreement between these two series of data is good.

When benzene was used as solvent 91.2% of the hydroperoxide remained after 414 min. at 109.4° C. whereas there was no decomposition in *n*-hexane in three hours at 109.4° C. Evidently the decomposition is much slower in solvents that less readily lose a hydrogen atom.

Derivation of Kinetic Equations

The decomposition of cumene hydroperoxide thermally is not a simple first order reaction. When styrene is used as solvent an analysis of the steps in polymerization may reveal the cause of this. There are four stages; initiation, propagation, transfer, and termination. These may be expressed by the following equations.

1. Initiation

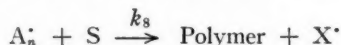
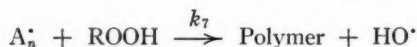
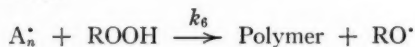


2. Propagation



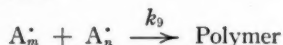
A_n^{\cdot} is a growing polymer radical of n monomer units.

3. Transfer



S is solvent.

4. Termination



These equations assume that the values of k_5 , k_6 , k_7 , k_8 , and k_9 are independent of the nature of the end group and of the number of monomer units attached to the growing polymer radical. This assumption appears to be valid when the number of units is large. At a steady state, the rate of formation of any radical is equal to its rate of disappearance. Thus for the RO^{\cdot} radicals:

$$k_1(P) + k_6(P) \sum_{n=1}^{n=r} A_n^{\cdot} = k_2(RO^{\cdot})(M) \quad (P) = \text{conc. ROOH} \quad (1)$$

Similar equations may be formed in the HO^{\cdot} , X^{\cdot} , A_1^{\cdot} , A_2^{\cdot} , A_r^{\cdot} radicals as follows:

$$k_1(P) + k_7(P) \sum (A_n^{\cdot}) = k_3(HO^{\cdot})(M) \quad (2)$$

$$k_8(S) \sum (A_n^{\cdot}) = k_4(X^{\cdot})(M) \quad (3)$$

$$k_2(RO^{\cdot})(M) + k_3(HO^{\cdot})(M) + k_4(X^{\cdot})(M) = k_5(A_1^{\cdot})(M) + (k_6 + k_7)(A_1^{\cdot})(P) + k_8(A_1^{\cdot})(S) + k_9(A_1^{\cdot}) \sum (A_n^{\cdot}) \quad (4)$$

$$k_5(A_1^{\cdot})(M) = k_5(A_2^{\cdot})(M) + (k_6 + k_7)(A_2^{\cdot})(P) + k_8(A_2^{\cdot})(S) + k_9(A_2^{\cdot}) \sum (A_n^{\cdot}) \quad (5)$$

$$k_5(A_{r-1}^{\cdot})(M) = (k_6 + k_7)(A_r^{\cdot})(P) + k_8(A_r^{\cdot})(S) + k_9(A_r^{\cdot}) \sum (A_n^{\cdot}) \quad (r + 3)$$

Adding Equations (1) to $(r + 3)$

$$\sum A_n^{\cdot} = \left(\frac{2k_1(P)}{k_9} \right)^{\frac{1}{2}} \quad (1')$$

$$\text{But } \frac{-d(P)}{dt} = k_1(P) + (k_6 + k_7)(P) \sum (A_n^{\cdot}) \quad (2')$$

Substituting (1') in (2'):

$$\begin{aligned} \frac{-d(P)}{dt} &= k_1(P) + (k_6 + k_7) \left(\frac{2k_1(P)^3}{k_9} \right)^{\frac{1}{2}} \\ &= k_1(P) + K(P)^{3/2}, \text{ when } K = (k_6 + k_7) \left(\frac{2k_1}{k_9} \right)^{\frac{1}{2}} \quad (3') \end{aligned}$$

Thus assumption of Reactions 1 to 4 leads to a kinetic equation for the disappearance of cumene hydroperoxide involving the sum of a first and a three-halves order reaction. The deviation from first order is caused by the radical-induced decomposition or chain transfer step.

Integrating (3'):

$$\ln(1 + k/(P)^{\frac{1}{2}}) - \ln(1 + k/(P_0)^{\frac{1}{2}}) = \frac{k_1 t}{2}, \text{ where } k = k_1/K. \quad (4')$$

For two different values of P_0 , at any time t

$$\begin{aligned} \ln(1 + k/(P_1)^{\frac{1}{2}}) &= \ln(1 + k/(P_2)^{\frac{1}{2}}) + \ln C \\ 1/(P_1)^{\frac{1}{2}} &= C/(P_2)^{\frac{1}{2}} + (C - 1)/k. \quad (5') \end{aligned}$$

If these equations describe the polymerization accurately a plot of $1/(P_1)^{\frac{1}{2}}$ versus $1/(P_2)^{\frac{1}{2}}$ should give a straight line with slope C and intercept equal to $(C-1)/k$, from which a value k may be calculated. A plot of $\log(1 + k/(P)^{\frac{1}{2}})$ versus t should give a straight line with a slope of $k_1/2 \times 2.30$. If k_9 is known then $k_6 + k_7$ may be determined.

It should be noted that k_s , the rate constant for propagation, does not appear in Equation (3'). Therefore Equations (3'), (4'), and (5') should hold for nonpolymerizing solvents that are reactive to RO^\cdot and OH^\cdot radicals if the same radical is produced by the reaction of RO^\cdot or OH^\cdot and the solvent. These equations are similar to those developed by Nozaki (19) for the decomposition of benzoyl peroxide in various solvents. The rate constants for transfer and termination would likely differ greatly from those obtained with styrene as solvent owing to the much shorter "chain" length and greater influence of terminal groups. The rate of decomposition in a polymerizing solvent is influenced by two factors. There is appreciable shrinkage during conversion of styrene to polystyrene; this causes a change in concentration of peroxide other than that due to the decomposition. In addition there is the question of whether polystyrene should be considered as solvent for the hydroperoxide or inert. The disappearance of hydroperoxide with an accompanying change in volume is not amenable to simple analyses, so further work was confined to mixed solvents in which both factors could be minimized.

Decomposition of Cumene Hydroperoxide in Mixed Solvents

The data collected, using styrene-benzene mixed solvent, are in Tables III and IV. The values of $1/(P_1)^{\frac{1}{2}}$ and $1/(P_2)^{\frac{1}{2}}$ are plotted in Fig. 5 while in Fig. 6 the values of $\log(1 + k/(P)^{\frac{1}{2}})$ are plotted against time. The curvature of Curves 5 and 6 of Fig. 6 is due possibly to depletion of the styrene in the dilute

TABLE III
DECOMPOSITION OF CUMENE HYDROPEROXIDE IN MIXED SOLVENTS

<i>t</i> , sec.	(<i>P</i> ₁)*, moles/l.	(<i>P</i> ₂)*, moles/l.	1/(<i>P</i> ₁) ^{1/2}	1/(<i>P</i> ₂) ^{1/2}	1 + <i>k</i> /(<i>P</i> ₁) ^{1/2}	1 + <i>k</i> /(<i>P</i> ₂) ^{1/2}
<i>Temperature 90.9° C. Styrene/benzene, 1 mole/4 moles</i>						
0.00 × 10 ⁴	9.96 × 10 ⁻³	4.70 × 10 ⁻³	10.02	4.61	2.645	1.755
0.24	9.90	4.64	10.05	4.64	2.650	1.760
0.48	9.82	4.60	10.09	4.66	2.655	1.765
0.72	9.78	4.54	10.11	4.69	2.660	1.770
0.96	9.74	4.47	10.13	4.73	2.660	1.775
1.44	9.67	4.39	10.16	4.77	2.665	1.780
1.92	9.62	4.29	10.19	4.83	2.670	1.790
2.40	9.49	4.23	10.26	4.86	2.680	1.795
3.96	8.73	3.89	10.70	5.07	2.755	1.830
5.40	8.62	3.68	10.76	5.21	2.765	1.855
8.64	8.06	3.45	11.13	5.38	2.825	1.885

Temperature 109.8° C. Styrene/benzene, 1 mole/4 moles

<i>t</i> , sec.	(<i>P</i> ₁)*, moles/l.	(<i>P</i> ₂)*, moles/l.	1/(<i>P</i> ₁) ^{1/2}	1/(<i>P</i> ₂) ^{1/2}	1 + <i>k</i> /(<i>P</i> ₁) ^{1/2}	1 + <i>k</i> /(<i>P</i> ₂) ^{1/2}
0.00 × 10 ³	9.22 × 10 ⁻³	4.53 × 10 ⁻³	10.41	4.70	4.34	2.505
1.80	8.94	4.35	10.58	4.79	4.38	2.535
3.60	8.78	4.16	10.67	4.90	4.42	2.570
5.40	8.50	4.06	10.85	4.96	4.48	2.585
7.20	8.30	3.92	10.98	5.05	4.52	2.615
9.00	7.87	3.75	11.27	5.16	4.60	2.660
10.80	7.63	3.69	11.45	5.20	4.66	2.665
12.60	7.49	3.57	11.55	5.29	4.70	2.695
14.40	7.41	3.44	11.61	5.39	4.72	2.725
16.20	7.25	3.38	11.75	5.44	4.76	2.740
18.00	7.09	3.31	11.88	5.50	4.80	2.760

Temperature 90.0° C. Styrene/benzene, 1 mole/9 moles

<i>t</i> , sec.	(<i>P</i> ₁)*, moles/l.	(<i>P</i> ₂)*, moles/l.	1/(<i>P</i> ₁) ^{1/2}	1/(<i>P</i> ₂) ^{1/2}	1 + <i>k</i> /(<i>P</i> ₁) ^{1/2}	1 + <i>k</i> /(<i>P</i> ₂) ^{1/2}
0.00 × 10 ⁴	9.64 × 10 ⁻³	4.56 × 10 ⁻³	10.19	4.68	6.51	3.54
1.10	9.37	4.41	10.33	4.76	6.60	3.58
2.15	9.12	4.30	10.48	4.83	6.68	3.62
6.50	8.25	3.86	11.01	5.09	6.97	3.76
8.65	7.99	3.69	11.20	5.21	7.07	3.82
15.10	7.27	3.39	11.73	5.44	7.36	3.95
17.30	7.15	3.29	11.82	5.52	7.41	3.99
25.90	6.70	2.99	12.21	5.79	7.63	4.14
28.10	6.60	2.94	12.30	5.94	7.66	4.22

* These values were calculated using the actual volume of the solution at the time of sampling.

solution used. The extrapolations in Fig. 5 are uncertain and the decomposition could not be followed further owing to formation of polystyrene with resulting shrinkage of the volume, increase in viscosity, and decrease in the styrene-to-benzene ratio.

From the data in Table IV the value of the rate constant for the unimolecular decomposition of cumene hydroperoxide into RO· and OH· radicals was calculated and found to be $2.7 \times 10^{12} e^{-30,400/RT}$ sec.⁻¹ between 90.9° and

TABLE IV
 RATE CONSTANTS IN MIXED SOLVENTS

Temperature, °C.	(P_0) moles/l.	k_1	$k_6 + k_7$, (moles/l) ⁻¹ sec. ⁻¹ *
90.9	9.96×10^{-3}	1.50×10^{-6} sec. ⁻¹	13.5
90.9	4.70×10^{-2}	1.50×10^{-6}	13.5
109.8	9.22×10^{-3}	1.20×10^{-6}	21.0
109.8	4.53×10^{-2}	1.20×10^{-6}	21.0

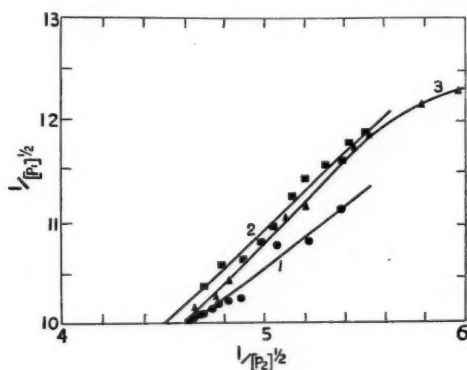


FIG. 5. Plot of $1/P^{1/2}$ at constant times for two initial values of cumene hydroperoxide concentration P . Curve 1. At 90.9°C . in styrene-benzene, molar ratio 1:4. Curve 2. Same at 109.8°C . Curve 3. At 90.0°C . in styrene-benzene, molar ratio 1:9.

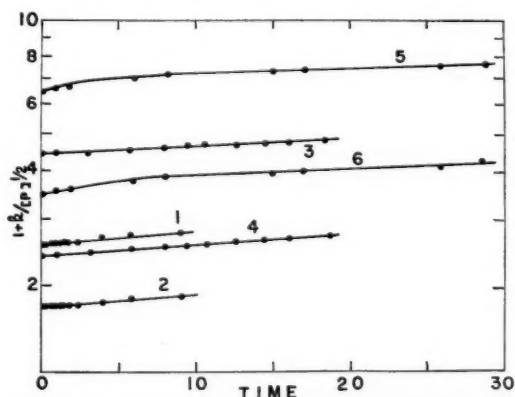


FIG. 6. Plot of $\log(1 + k/P^{1/2})$ versus time. Curve 1; 9.96×10^{-3} molar solution of cumene hydroperoxide (concentration P) in styrene-benzene, molar ratio 1:4, at 90.9°C . Curve 2; 4.70×10^{-2} molar solution in same. Curve 3; 9.22×10^{-3} molar solution in same at 109.8°C . Curve 4; 4.53×10^{-2} molar solution in same. Curve 5; 9.64×10^{-3} molar solution in styrene-benzene, molar ratio 1:9, at 90.0°C . Curve 6; 4.56×10^{-2} molar solution in same. Curves 1, 2, 5, and 6; time is seconds $\times 10^{-4}$; Curves 3 and 4; seconds $\times 10^{-3}$.

109.8° C. The value of the energy of activation (30.4 kcal. per mole) is higher than the previous estimate because the latter included the effect of the radical-induced decomposition.

Assuming (2) a termination rate constant

$$k_9 = 3.07 \times 10^8 \times e^{-2800/RT} \text{ (mole/l.) sec.}^{-1}$$

the value of $k_6 + k_7$ is $1.00 \times 10^8 e^{-6500/RT} \text{ (mole/l.)}^{-1} \text{ sec.}^{-1}$.

Attempts to gain accuracy by following the reaction further must also take into account changes in the concentration of styrene in benzene. That this is appreciable is shown in comparing the initial rate of decomposition of cumene hydroperoxide in Table III. When the more dilute solution of styrene is used the initial rate, expressed as a percentage of the original concentration per unit of time, is very nearly independent of the concentration, in contrast with the results using the solvent richer in styrene, where the rate is faster when the initial hydroperoxide concentration is greater.

This effect is accentuated when pure benzene is used as a solvent. The results are in Table V.

TABLE V
DECOMPOSITION OF CUMENE HYDROPEROXIDE IN BENZENE AT 90.9° C.

Time, hr.	% unreacted cumene hydroperoxide	
	2.210×10^{-2} molar solution	1.102×10^{-1} molar solution
0.0	100	100
96	83.8	92.6
168	34.4	81.7

The results indicate the autocatalytic effect of increase in rate of decomposition with time but in addition show a relatively more rapid rate of decomposition in dilute solution than in concentrated solution. The cause of this was not elucidated. This effect might be related to some of the results obtained during emulsion copolymerization using this hydroperoxide. It was decided, however, to defer any further consideration and to include it in the study of the catalyzed decomposition of cumene hydroperoxide, which will be a separate report.

Analysis of the Products of Decomposition in Cumene

To obtain information on the reactions of the radicals formed from cumene hydroperoxide an attempt was made to analyze the products formed in cumene at 90.5° C. Cumene was chosen as the solvent rather than styrene because of the difficulty of identifying the hydroperoxide fragments in the larger polystyrene molecule.

Numerous preliminary experiments were considered unsuitable because of the rapid temperature increase during the autocatalytic stage. This was

overcome by increment addition of the hydroperoxide. The reaction products were prepared on a macro scale by reacting 0.2 mole of cumene hydroperoxide in 1.8 mole of cumene. Distilled cumene, 1.6 moles, was added to a 500 ml. round-bottomed flask provided with a thermometer well and fitted with a 100 ml. graduated addition flask, the take-off tube of which was connected to a trap immersed in a dry ice - ethanol bath and a gas measuring set. The flask was placed in a dry ice - ethanol bath and subjected to the same treatment as the solutions in the rate studies with regard to evacuation and nitrogen flushing, except that an additional nitrogen flushing was included and the cumene was left under a pressure of $2\frac{1}{2}$ p.s.i. of nitrogen. The flask was placed in a constant temperature bath at 90.5°C . and after equilibrium was obtained was vented to the atmosphere. A solution containing 0.2 mole of distilled cumene and 0.2 mole of the hydroperoxide was added to the addition flask, and the top was closed with a glass stopper. Enough of the solution was added to give an initial hydroperoxide concentration of 2%. After about two hours a slight increase in temperature was noticed. The solution was added in increments over a five-hour period so that the temperature of the reaction mixture was held at $90.5 \pm 1.0^{\circ}\text{C}$. The reaction was allowed to continue for two hours after all the cumene hydroperoxide solution had been added.

The products were then subjected to analysis. The volume of liberated gas was found to be very small and probably could be accounted for fully by the increased vapor pressure of the reaction mixture caused by the presence of volatile products. No liquid was condensed in the trap; this demonstrated that the formation of very volatile products does not occur. The liquid in the flask consisted of two layers—a very small lower watery layer (2 ml.) and a much larger upper oily layer. Determination of the residual peroxide in the upper layer revealed that less than 0.1% of the initial weight of CHP remained. The upper layer was also analyzed for acetophenone (1.90 gm. or 0.016 mole) and phenol (1.70 gm. or 0.018 mole), using an ultraviolet absorption method. These values were corrected for the absorption caused by the cumene, which is probably the principal interfering compound at the wave lengths used ($241\text{ m}\mu$ for acetophenone and $273\text{ m}\mu$ for phenol). Both layers were then distilled under 3 mm. of pressure in a 15 in. unpacked column until the vapor temperature reached 40°C . This distillate was fractionated at atmospheric pressure in a 100 plate Podbielniak column at a 30 : 1 reflux ratio, using stripped diethylbenzene chaser, which boiled at 178°C . From this distillation were recovered the products shown in Table VI.

The residue from the vacuum distillation was distilled at 2 mm. pressure until the vapor temperature reached 60°C . This distillate was extracted twice with 10 ml. portions of water at 70°C . to remove the phenol. The water was removed from the extract under vacuum at room temperature. Phenol, 1.40 gm. (0.015 mole), which melted at 40°C ., was recovered. The non-extracted portion probably contained acetophenone and α,α' -dimethylbenzyl alcohol, the weight of which was calculated by difference (4.80 gm. or 0.035 mole). The ratio of alcohol to acetophenone agrees approximately with the

TABLE VI
ANALYSIS OF DECOMPOSITION PRODUCTS

Product	Weight		B.p. at 760 mm.	η_{sp}^D
	Gm.	Moles		
Acetone	7.55	0.130	56.2	1.3571
Water	1.55	0.087	100.0	—
Cumene	206.2	0.715	152.2	1.4889
Higher boiling compounds	15.00	—	—	—

ratio given for these impurities in a typical sample of commercial cumene hydroperoxide where they are formed by its decomposition during manufacture. This agreement tends to support the validity of the value given here for the alcohol. A summary of the weights of all the products identified is presented in Table VII.

TABLE VII
ANALYSIS OF PRODUCTS OF DECOMPOSITION

Product	No. of moles per mole hydroperoxide
Acetone	0.65
Acetophenone	0.08
Phenol	0.09
Water	0.43
α, α' -Dimethylbenzyl alcohol (calculated)	0.17
Cumene (reacted)	0.42

These are not all the products in the reaction mixture but they are the only ones that have been identified. However it is not very likely that the side reactions that take place in cumene are important from the standpoint of polymerization initiation. No acetone was isolated from the decomposition of 0.2 mole of cumene hydroperoxide in 1.8 moles of styrene at 90.5° C. It is not feasible to analyze for acetophenone in styrene because of its strong absorption at the wave length used. However, in view of the work done on the macro decomposition in cumene, no formation of acetophenone would be expected. It seems that the $C_6H_5C(CH_3)_2O\cdot$ radicals are able to initiate polymerization before they can break down to acetone and a phenyl radical.

Effect of Shortstops

It was thought that some concept of the mechanism by which a shortstop brings a halt to the polymerization reaction could be elucidated by a study of the effect of shortstops on the thermal decomposition of cumene hydroperoxide in styrene. Subsequently results were obtained *in vacuo* for the two

shortstops used currently in the low temperature recipes, 2,5-ditertiarybutylhydroquinone and 2,4-dinitrochlorobenzene, and other related compounds known to be effective (11). An initial cumene hydroperoxide concentration of 0.2 mole per kgm. of final solution was chosen and 0.02 mole per kgm. of solution of a shortstop or related compound was added to the hydroperoxide solution in styrene, previously cooled to a temperature just above its freezing point and kept at this temperature or lower until the reaction tubes were immersed in the bath at 90.6° C. In addition to the results given in Table VIII an attempt was made to study the effect of hydroquinone, which

TABLE VIII
UNREACTED CUMENE HYDROPEROXIDE, % OF INITIAL WEIGHT

Reaction time, min.	No shortstop	2,5-Ditertiary-butylhydroquinone	2,4-Dinitrochlorobenzene	2,6-Ditertiary-butyl-4-methylphenol	Phenyl- β -naphthylamine
0.00*	100.0	90.0†	90.0‡	99.5	97.5
20.00	88.3	88.4	100.0	85.1	93.3
40.00	77.8	85.4	90.0	88.9	88.1
60.00	67.0	81.5	80.0	82.6	82.6
80.00	51.9	79.0	80.9	78.4	76.1
100.00	34.0	76.2	79.2	68.2	68.3
120.00	16.4	73.5	78.0	58.7	61.2
140.00	3.8	70.5	74.1	48.2	52.6
160.00	—	66.8	71.2	42.6	37.1
180.00	—	65.1	68.3	38.8	32.7
200.00	—	62.9	65.1	34.0	32.7
220.00	—	58.6	62.0	29.0	— §
240.00	—	46.0	44.1	— §	— §

† Eastman Kodak Technical.

‡ Monsanto recrystallized.

§ Too viscous to analyze.

* Samples removed from solution at -10° C.

is the shortstop used to stop persulphate-initiated polymerization, and *p*-benzylaminophenol and *p*-nitrobenzeneazo-3,4,5-trihydroxyphenol, photographic develops that were found to stop the reaction satisfactorily in the polymerization laboratory (11). These attempts were unsuccessful because of the insolubility of hydroquinone in the solution and the interfering color of the latter two compounds and/or their derivatives which completely masks the end point of the titration in the method of analysis used.

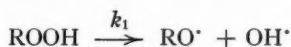
From these results it would seem that 2,5-ditertiarybutylhydroquinone reacts with equimolar amounts of cumene hydroperoxide in a styrene solution at -10° C. The resulting products are powerful retarders for the decomposition of the hydroperoxide in styrene at elevated temperatures. 2,4-Dinitrochlorobenzene, 2,6-ditertiarybutyl-4-methyl phenol and phenyl- β -naphthylamine do not react under these conditions mentioned but they do retard the decomposition in styrene at elevated temperatures. 2,4-Dinitrochlorobenzene is a more powerful retarder than is either of the other two.

Discussion

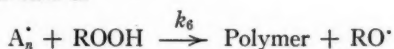
Cumene hydroperoxide would appear to enter into two phases of polymerization. The first is the formation of free radicals either by unimolecular dissociation, or by reaction with solvents or activators, or by chain transfer with solvents or a growing polymer radical. These free radicals in turn react with a monomer to start a growing polymer chain. The second phase is the stopping of the polymerization reaction by terminating the growing polymer chains and destroying the residual initiator or otherwise rendering it inactive.

The thermal decomposition of cumene hydroperoxide in a solution of monomer such as styrene is the sum of a first order and a three-halves order reaction. The first order reaction arises from the unimolecular decomposition. The three-halves order reaction arises from the radical-induced chain transfer reaction. In pure styrene the reaction rapidly becomes autocatalytic.

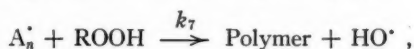
The relative amounts of peroxide consumed in the two reactions is of interest since the ratio of the two types of decomposition enters into the molecular weight distribution studies of the resulting polymers. If Reaction (I) is



and Reaction (II) is the sum of



and



then $\left. \frac{-d(P)}{dt} \right|_{(I)} = k_1 (P)$ (rate of disappearance of hydroperoxide by Reaction (I))

$$\begin{aligned} \left. \frac{-d(P)}{dt} \right|_{(II)} &= (k_6 + k_7) (P) \Sigma (\text{A}_n^\bullet) \text{ (disappearance by Reaction (II))} \\ &= (k_6 + k_7) \left(\frac{2k_1}{k_9} \right)^{\frac{1}{2}} (P)^{3/2} \\ &= K (P)^{3/2}. \end{aligned}$$

$$\text{Therefore } \frac{-d(P)|_{(I)}}{-d(P)|_{(II)}} = \frac{k_1}{K} \cdot \frac{1}{(P)^{\frac{1}{2}}} \quad \text{or} \quad \frac{-\Delta(P)|_{(I)}}{-\Delta(P)|_{(II)}} = \frac{k}{(P)^{\frac{1}{2}}}$$

$$\text{or } \frac{\Delta(P)|_{(II)}}{\Delta(P)|_{(I)}} = P^{\frac{1}{2}}/k.$$

From this analysis it can be seen that the relative amount of cumene hydroperoxide consumed by transfer should increase with increasing concentration and decreasing temperature since k decreases with decreasing temperature.

However, not all of the radicals need start polymerization. None do in the absence of monomers. Following the initial split of the cumene hydroperoxide into $\text{C}_6\text{H}_5\text{C}(\text{CH}_3)_2\text{O}^\bullet$ radicals and OH^\bullet radicals, a chain of events may occur.

Under the conditions of the experiments in cumene most of the $\text{C}_6\text{H}_5\text{-C}(\text{CH}_3)_2\text{O}^\cdot$ radicals lose a radical and form a ketone; the tendency is for the radical to lose the phenyl radical and form acetone in preference to losing a methyl radical and forming acetophenone. Kharasch (12) suggests that the mechanism of these steps is different.

The OH^\cdot radicals are not accounted for as completely. Probably some abstract a hydrogen atom from cumene to form water. The cumene disappears in about the same molar proportions as the water formed. Others react with free phenyl radicals to yield phenol. Possibly the α,α' -dimethylbenzyl alcohol may be accounted for by the recombination of the $\text{C}_6\text{H}_5\text{C}(\text{CH}_3)_2^\cdot$ and OH^\cdot radicals. Its formation from the $\text{C}_6\text{H}_5\text{C}(\text{CH}_3)_2\text{O}^\cdot$ radical by the abstraction of a hydrogen atom from cumene does not seem to be as probable. Other unidentified products remained and may account for the residual portion of the material balance. The products appeared to be higher hydrocarbons, possibly with hydroxyl groups.

The stopping of polymerization appears to occur by two distinct mechanisms. 2,5-Ditertiarybutylhydroquinone and 2,4-dinitrochlorobenzene, which are each considered good stopping agents, exemplify the two types. The former reacts quantitatively and rapidly with the cumene hydroperoxide at -10°C . thereby destroying the initiator system. Presumably the hydroquinone is converted to an inactive semiquinone or quinone. The 2,4-dinitrochlorobenzene on the other hand acts as a polymerization retarder by effectively inhibiting the decomposition of cumene hydroperoxide. While the mechanism of this action is not clear it may be related to systems described by Bovey and Kolthoff (4) in which free radicals are consumed and normal propagation is thereby eliminated. In the same category but less effective as inhibitors are the other soluble shortstops studied. Lack of oil solubility is, of course, a limitation that excludes such familiar stopping agents as hydroquinone and sodium sulphide.

The desirable shortstop would seem to be one that was sufficiently oil soluble to enter the emulsion droplet and react with the residual initiator and yet be sufficiently soluble in the aqueous phase to diffuse rapidly from the source of stopping agent to the emulsion droplet.

The thermal decomposition of cumene hydroperoxide requires such elevated temperatures that it has no role in low temperature polymerization reactions at or below 5°C . Catalytic and induced decomposition must account for all of the initiation of polymerization obtained. Likewise in the absence of a catalyst little effect would be expected from any residual initiator left in the polymer while the latter was being coagulated and dried for short periods at 70° to 80°C . The effect might become noticeable during longer drying periods or during the short periods at much higher temperatures (150°C .) as encountered during the degradation of the polymer during compounding and curing. In view of these findings only the catalytic decomposition will receive further study.

Acknowledgments

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THE EFFECT OF CHAIN LENGTH ON THE INTRINSIC VISCOSITY - TEMPERATURE COEFFICIENT OF LINEAR HIGH POLYMERS¹

BY L. H. CRAGG AND J. E. SIMKINS²

Abstract

Careful measurements of the intrinsic viscosities of three fractions of polystyrene, ranging in molecular weight from 160,000 to 1,350,000, have been made over a 40° C. range of temperature in a good solvent (toluene) and a poor one (toluene - 30% 1-butanol). These, together with similar but somewhat less accurate measurements with five fractions of GR-S, show clearly that solvent effects on the intrinsic viscosity are strongly dependent on molecular weight for linear flexible polymers, and give additional support to the concept that the differences in the intrinsic viscosity of such a polymer in good or poor solvents are due primarily to differences in the looseness or tightness of coiling of its flexible molecules in solution.

Although the viscosity of a dilute solution of a high polymeric substance is the viscosity of the solvent modified by the presence of the solute, the extent of the modification is out of all proportion to the number of solute molecules present. The disproportionate effect of these molecules may be attributed to their unusual size; for linear macromolecules of a given homologous polymeric series the effect is greater the longer the molecules are, that is, the higher their molecular weight. This effect of the solute molecules is isolated when the specific viscosity, $\eta_r - 1$, or better the reduced viscosity, $\frac{\eta_r - 1}{c}$, is calculated, and the secondary effect of solute molecules on each other is eliminated in the intrinsic viscosity, $[\eta] \equiv \lim_{c \rightarrow 0} \frac{\eta_r - 1}{c}$. Thus the intrinsic viscosity should be a function of chain length. For many linear polymers the relation is given by the empirical equation $[\eta] = KM^a$, where M is the molecular weight of the polymer if all its molecules are identical or its "viscosity-average molecular weight" if they are not, and K and a are empirical constants (7). Unfortunately, even with a given polymer, K and a must be evaluated for each different solvent, for the intrinsic viscosity (unlike the limiting value of the reduced osmotic pressure, $\left(\frac{\pi}{c}\right)$) varies from solvent to solvent. This effect must be due to solvent-solute interaction of some kind. It was at one time attributed to solvation of the polymer molecules and to the change in their effective size and shape resulting from the addition of a solvation sheath. More recently the belief has grown that, although this effect of solvation may make its contribution (and undoubtedly does in many

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systems (6, 23)), the effect of solvent on flexible polymer molecules is largely due to its influence on the shape that the polymer molecules themselves assume in solution (3, 15, 17, 21).

A flexible linear polymer molecule may be thought of as a chain of segments jointed together by more or less flexible links. In an "energetically favorable" (3) solvent, segments are attracted more by the surrounding solvent than they are by other segments in the chain, and as a result the flexible molecule tends to be stretched out. Such a solvent we shall term "good." By contrast, in an energetically unfavorable (or "poor") solvent the forces between segments are dominant and the polymer molecule draws in upon itself, assuming a randomly coiled configuration; the poorer the solvent the more tightly coiled is the molecule. Thus the intrinsic viscosity is greater in the good solvent.

Of course by "shape" (or "configuration") is meant the average shape, for the molecules in consequence of their flexibility are undergoing what has been called "internal Brownian movement" and are changing shape, and hence the degree of coiling, continuously and rapidly. The most probable shape lies between the elongated one in a good solvent and the tightly coiled one in a poor solvent. Raising the temperature and therefore the vigor of this internal Brownian movement would favor the more probable configuration, and hence decrease the intrinsic viscosity of a polymer in a good solvent and increase it in a poor solvent. By adding a nonsolvent to a solvent it should be possible to change gradually from a good solvent to a poor solvent and therefore to change from a high to a low intrinsic viscosity and from a negative to a positive slope of the intrinsic viscosity vs. temperature curve.

These and other predictions have stood the test of experiment (2, 3, 4, 10). In a paper describing some of the experimental vindications (10) another consequence of the theory was suggested, namely, that "one would expect that much greater effects would be observed with very long molecules than with short ones" (or, in other words, the changes in intrinsic viscosity and the slope that were mentioned above should be more marked with the larger molecules). Subsequently, fractionated samples of GR-S and of polystyrene, varying sufficiently in molecular weight, became available, and the expectation could be tested.

Experimental

1. MATERIALS

Solvents and Nonsolvents

Benzene — (Steel Company of Canada), ASTM Industrial Grade, redistilled and dried over sodium; b.p. 80.5° C.

Toluene— (Steel Company of Canada), "nitration grade," redistilled; b.p. 110° C.

Methanol — (Carbide and Carbon Chemical Company), dried over calcium oxide and redistilled; b.p. 63.7° C.

1-Butanol— (Eastman Kodak), Reagent Grade.

Polymers

GR-S

The unfractionated material was a normal plant sample obtained in bale form from Polymer Corporation, Limited, Sarnia, on July 9, 1946. It has been described elsewhere (10); no change in intrinsic viscosity was observed with time. Its viscosity-average molecular weight* was 224,000.

Five fractions of molecular weight (\bar{M}_v)* 631,000, 561,000, 274,000, 124,000, and 21,500, respectively, were obtained from D. F. Switzer (24, 25). They were numbers 1, 2, 7, 10, and 14 of the 14 fractions obtained by him in a primary fractionation by precipitation from 2% solution in benzene, using methanol as precipitant. They were obtained as solutions (approximately 0.5% in polymer) in benzene containing a small proportion of methanol (always less than 1.8%). Subsequent work by Switzer has proved that the presence of these small amounts of methanol does not affect the values of intrinsic viscosity by more than the experimental error, $\pm 2\%$.

Polystyrene

A sample of unfractionated polymer and of three fractions from it were obtained from Monsanto Chemical Company. These materials were prepared as follows:

"Commercial monostyrene containing about 10 ppm. of *paratertiary butyl catechol* was polymerized in bulk at 93° C. without the use of catalyst. After 12 hours, 15% conversion was reached and the reaction was stopped by cooling to 20° C. in less than 15 minutes. 0.1% hydroquinone was then added. The polymer was isolated by dripping the solution of polymer in monomer into 10 volumes of methanol; it was filtered and washed with methanol again. The polymer was then dissolved in methyl ethyl ketone (MEK) to make a 2% solution and then precipitated in the form of a porous thread having a high surface/weight ratio by running a fine stream of the polymer solution into 10 volumes of stirred methanol.

"The twice precipitated material was then dissolved in methyl ethyl ketone to make a 1.5% solution; methanol was added until the solution became cloudy. The temperature was raised until all the material was again dissolved, then lowered slowly to precipitate a fraction as a gel. This gel was drawn off, dissolved in MEK and again precipitated with a high surface/weight ratio in ethanol.

"Altogether, 40 fractions were obtained. The fractions were vacuum dried at room temperature and stored at room temperature under CO₂ in the absence of light."

The samples used in this study are of fractions 3, 21, 35, and of the original polymer. The molecular weights, determined from intrinsic viscosities

* Calculated from the values of $[\eta]$ at 25° C., using the equation of Scott, Carter, and Magat (20).

calibrated by light scattering measurements, were given as 1,350,000, 525,000, 160,000, and 730,000 respectively (the last a viscosity-average).

2. APPARATUS

The viscometers used were of the Ostwald-Cannon-Fenske type (ASTM 50); they had been carefully calibrated over the whole range of flow times encountered. The various temperatures of measurement were maintained in constant-temperature baths regulated to $\pm 0.02^\circ\text{C}$. Specially designed viscometer clamps incorporating standard-taper pins made it possible to transfer a viscometer from bath to bath conveniently and yet be sure that the mounting in each bath was reproducibly vertical. After each such transfer the viscometer and contents were allowed to equilibrate for 10 min. before flow times were measured.

3. INTRINSIC VISCOSITY DETERMINATIONS

Flow times were measured as before (10) in duplicate or triplicate (or until agreement to ± 0.1 sec. was obtained), first at the lowest temperature and then at successively higher temperatures. Even in the polystyrene experiments, which involved successive measurements at five temperatures, there was neither significant evaporation nor degradation, as proved by the observation that the flow times measured at 25°C . after such a run were exactly the same as at its beginning. Concentrations of the solutions were calculated as weight per cent (grams solute per 100 gm. solution) and corrected to the usual units (grams per 100 ml.) at each temperature by multiplying by the density of the solution at that temperature. (Actually the density of the solvent was used but the error introduced by this approximation is negligible. The densities of the pure solvents at the various temperatures were taken from the literature (18); those for the mixed solvents were measured.)

With polystyrene solutions, the *concentration* was calculated from the measured weights of dry polymer and solvent used in preparing the solution.

With GR-S, the concentration could not be determined in this way because the fractions used were available only as solutions (from which they could not be separated by evaporation because of gel formation); it was therefore determined by evaporating a weighed amount of solution to constant weight at 70°C . This method is more precise than accurate but since we are concerned with relative values precision is more important than accuracy.

Intrinsic viscosities were determined from values of flow time and concentration, as follows:

(a) GR-S

Solutions in benzene were prepared of four concentrations from 0.05 to 0.4 gm. per 100 ml., values of the inherent flow time (9) $\frac{\ln t_r}{c}$ calculated, and the intrinsic flow time $[\eta]$ determined by extrapolation. Although kinetic

energy corrections were not applied, the viscometers had been calibrated and it was known that the intrinsic viscosity $[\eta]$ would differ from $[\iota]$ by not more than 3 or 4%.

Solutions in mixed solvent (benzene - 13.2% methanol) were prepared of only one concentration and the intrinsic viscosity calculated by the equation

$$[\iota] = \frac{8 (\iota_r^4 - 1)}{c} \quad (11)$$

This one-point method is known to be somewhat less accurate for the highest fractions of GR-S than for intermediate and low fractions or for unfractionated material (24, 25) but the error introduced in $[\eta]$ is still small.

(b) Polystyrene

All values of $[\eta]$ were obtained by extrapolation, using the values of inherent kinematic viscosity $\frac{\ln v_r}{c}$ (9) for three or four solutions ranging in concentration from 0.1 to 0.4 gm. per 100 ml.; the values of $\frac{\ln v_r}{c}$ were calculated from flow times by applying the kinetic energy correction. Because of these two refinements, and because with polystyrene the values of the concentration, c , were more accurate, the values of $[\eta]$ in the polystyrene study are more accurate than in the corresponding GR-S study.

Results

For GR-S, benzene is neither a very good nor a very poor solvent, for the $[\eta]$ vs. θ (temperature) curves are practically horizontal (10, 19). It can be made a poor solvent by the addition of a nonsolvent; at 18.3 vol. % methanol, precipitation begins at 15° C. for original polymer. Accordingly benzene-methanol containing 15 vol. % (13.2 wt. %) methanol was selected as a solvent that would be poor without being too poor. Intrinsic viscosities of the various fractions of GR-S and of the unfractionated material, as measured in this solvent at three temperatures, 15°, 35°, and 50° (or 55°) C., are shown graphically in Fig. 1. For comparison the variation of $[\eta]$ with temperature in pure benzene and in the mixed solvent is shown, for a high and a low fraction, in Fig. 2. (For lack of sufficient material, measurements in pure benzene could not be made with all fractions of GR-S. Thus with F-1 the intrinsic viscosity at 25° C. is that determined by Switzer (24, 25) and the curve is drawn in accordance with the general experience in this laboratory that the $[\eta]$ vs. θ line for GR-S and for GR-S fractions in benzene is always practically horizontal.) For polystyrene, toluene is a good solvent, giving a negative value of $\frac{d[\eta]}{d\theta}$, and toluene - 1-butanol containing 30% (by weight) of 1-butanol is a poor solvent (at 25° C. precipitation begins at ca. 38% butanol). The experimental data for the polystyrene-toluene and polystyrene-toluene-butanol systems at 25°, 35°, 45°, 55°, and 65° C. are given in Tables I and II and in Figs. 3 and 4. There is a definite suggestion that with the poor solvent the points lie on a curve slightly convex upwards, but the

evidence for the curvature is not sufficiently conclusive to offset the appeal of the straight line. (It should be noted, however, that working with polyisobutylene fractions in a poor solvent over a larger range of temperature — 0°

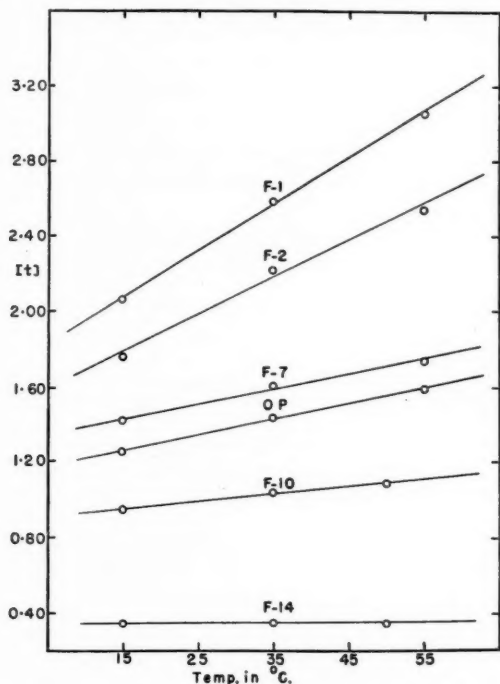


FIG. 1. Variation of intrinsic viscosity with temperature for GR-S (five fractions and the original polymer, OP) in benzene-methanol. The molecular weights (\bar{M}_v) are: OP 244,000, F-1 631,000, F-2 561,000, F-7 274,000, F-10 124,000, and F-14 21,500.

to 90° C. — Fox and Flory (16) have found experimental and theoretical evidence for a downward curvature.)

Discussion

The behavior of both GR-S and polystyrene, as indicated in these results, is just what was predicted. For example, the marked increase, with increasing molecular weight, of the slope $d[\eta]/d\theta$ in poor solvent that is evident in Figs. 1 to 4 is easily accounted for on the basis of uncoiling molecules: if an increase in temperature tends to uncoil the molecules in a poor solvent then even with the same degree of uncoiling the increase in linear dimension should be greater with the longer molecules.

Actually however one would expect the degree of uncoiling to be not the same but greater with the longer molecules. The flexibility of chain molecules

is by no means unlimited. Even the most flexible ones must do their bending gradually (like garden hose) and this puts a limit on the tightness of coiling that is possible. Furthermore this flexibility — or lack of it — is inherent in

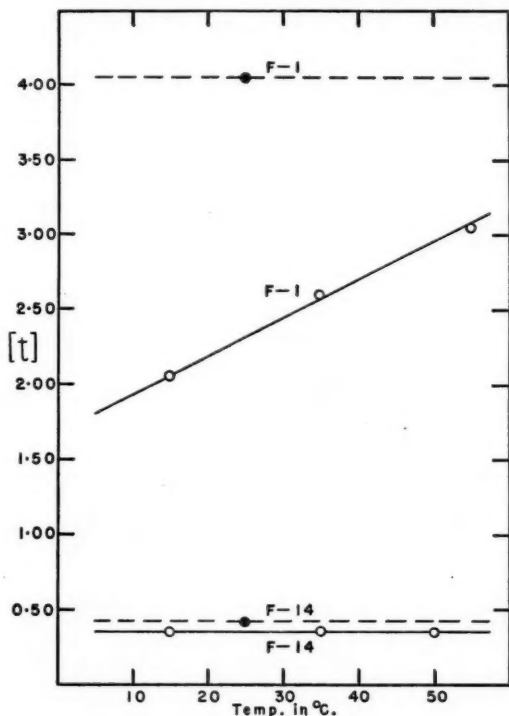


FIG. 2. Comparison of intrinsic viscosity - temperature behavior of high and low fractions of GR-S in good and poor solvents.

—●—●— in benzene.
—○—○— in benzene-methanol.

the structure of the chain and independent of its length. More precisely, the *intensive* flexibility (f. per unit length) of a long molecule is the same as that of a short molecule even though its *extensive* flexibility (f. for the molecule as a whole) is much greater. Accordingly, the ratio of the length of the molecule when extended to its length when coiled up should be greater for long molecules, and any effect resulting from uncoiling, whether as a consequence of an increase in temperature or of a change from poor to good solvent, should be *relatively* greater for the long molecules. In terms of our data, then, both the ratio $[\eta]_{\text{good}}/[\eta]_{\text{poor}}$ at any given temperature, and the relative slope $d[\eta]/d\theta \cdot [\eta]$ at

TABLE I

VARIATION OF INTRINSIC VISCOSITY WITH TEMPERATURE: POLYSTYRENE IN TOLUENE

Sample	\bar{M}_v	Intrinsic viscosity*				
		25° C.	35° C.	45° C.	55° C.	65° C.
F-3	1,350,000	2.30	2.29	2.28	2.29	2.28
F-21	525,000	1.32	1.31 ₅	1.32	1.32	1.30
F-35	160,000	0.57	0.57	0.57	0.56	0.57
Unfractd.	730,000	1.62	1.62	1.60	1.59	1.60

* Because of the great advantage in routine measurement of a one-point method for determining intrinsic viscosity, it is important to note that values of $[\eta]$ calculated from measurements of t , at only one concentration, $c = 0.20 \pm 0.01$ gm. per 100 ml. solution, using the modified Baker equation (11)

$$[\eta] = \frac{n(t_r^{1/n} - 1)}{c}$$

with a value of $n = 3$, differed from those recorded in the table by an amount that was in only one instance more, and was usually less, than the experimental error, ± 0.02 , of the extrapolated value.

TABLE II

VARIATION OF INTRINSIC VISCOSITY WITH TEMPERATURE: POLYSTYRENE IN TOLUENE-1-BUTANOL (30%)

Sample	\bar{M}_v	Intrinsic viscosity				
		25° C.	35° C.	45° C.	55° C.	65° C.
F-3	1,350,000	1.22	1.34	1.43	1.52	1.60
F-21	525,000	0.77	0.82	0.87	0.91	0.94
F-35	160,000	0.39	0.40	0.42	0.43	0.43
Unfractd.	730,000	0.90	0.98	1.04	1.11	1.14

TABLE III

COMPARISON OF THE BEHAVIOR OF HIGH, INTERMEDIATE, AND LOW FRACTIONS OF POLYSTYRENE

	F-3	F-21	F-35
\bar{M}_v	1,350,000	525,000	169,000
$[\eta]_{\text{good}}/[\eta]_{\text{poor}}$ at 25° C.	1.89	1.71	1.46
$d[\eta]/d\theta \cdot [\eta]_{25}^*$ in poor solvent*	0.0078	0.0058	0.0026

* These values are calculated from the straight lines drawn through the points of Fig. 3. The same trend would be observed if curved lines had been drawn and slopes determined at 25° C.

any temperature, should be greater for the high fractions than for the low ones. Values of these functions at 25° C. calculated from the data for polystyrene are given in Table III. These, too, are in accord with expectations.

Such excellent agreement with predictions does not, of course, establish the validity of the theory that the solvent affects the shape of the polymer molecule dissolved in it, but it certainly does add support to it. Other strong evidence

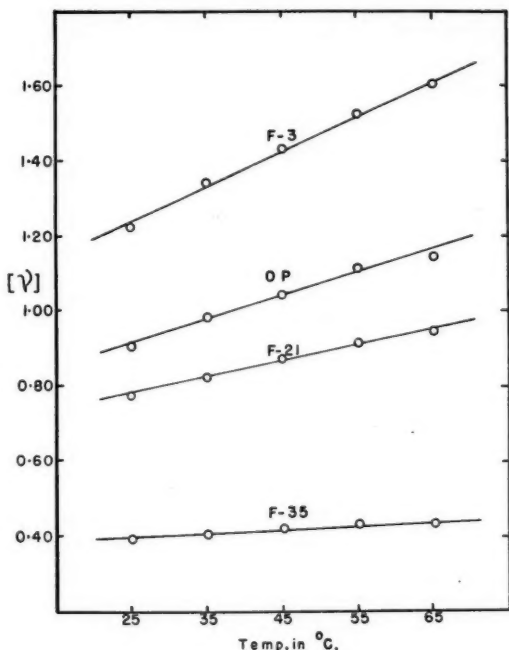


FIG. 3. Variation of intrinsic viscosity with temperature for polystyrene (three fractions and the original polymer) in toluene-butanol. The molecular weights (\bar{M}_v) are OP 730,000, F-3 1,350,000, F-21 525,000, and F-35 160,000.

has been accumulating from experiments of other kinds: viscosity (5, 6, 8), diffusion (1), angular dissymmetry of light scattering (13, 23, 27) and depolarization of scattered light (12, 14).

For this investigation the poor solvents used were synthetic ones prepared by mixing solvent and nonsolvent. Recently Fox and Flory (16) have described the viscosity behavior of fractions of polyisobutylene in several solvents over a range of temperatures. For polyisobutylene, benzene and toluene are "poor" solvents, the values of $d[\eta]/d\theta$ being positive; and, as in our systems involving synthetic poor solvents, the temperature effect on intrinsic viscosity is much more marked with the high fractions. Other examples may be in the literature but, if so, they are few in number. Indeed comparatively few polymer-solvent systems exhibiting a marked increase of intrinsic viscosity with temperature have been reported (probably because of

a natural preference for good solvents and because most work has been done with polymers of relatively low molecular weight) and in nearly all such reports the polymer was unfractionated.

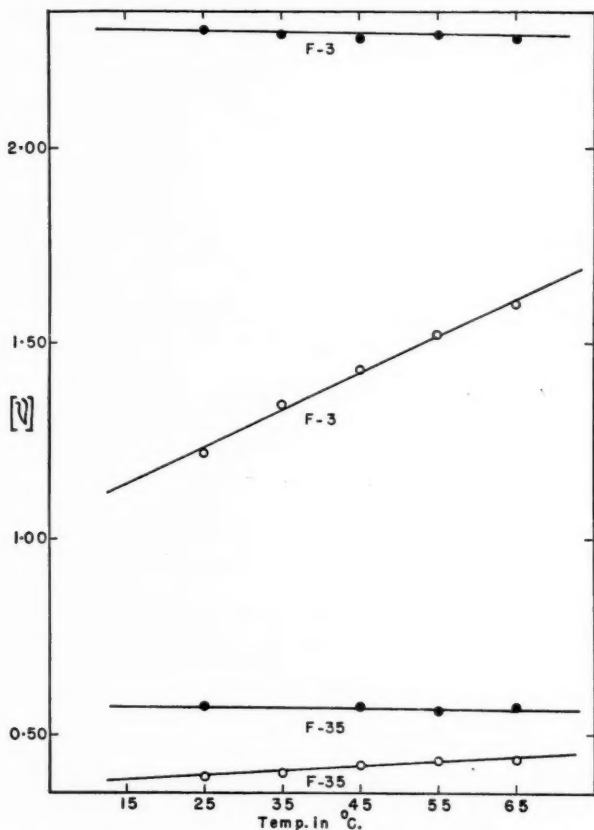


FIG. 4. Comparison of intrinsic viscosity-temperature behavior of high and low fractions of polystyrene in good and poor solvents.

—●—●— in toluene.
—○—○— in toluene-butanol.

The data of Fox and Flory for polyisobutylene also permit the calculation of values of $[\eta]_{\text{good}}/[\eta]_{\text{poor}}$, at 30° C., over a wide range of molecular weights with four combinations of the two poor solvents, benzene and toluene, and the two good solvents, cyclohexane and carbon tetrachloride (strictly these are "indifferent" solvents, for $d[\eta]/d\theta$ appears to be negligibly small). Again these values are greater for the higher fractions.

There is good reason to believe that the molecules of GR-S and of polystyrene (and of polyisobutylene) are much more flexible than those of, say, cellulose acetate or cellulose nitrate (22, 26). One would therefore expect lesser effects (particularly at lower molecular weights) with cellulose derivatives than those reported here, other things being equal. These effects are a consequence of the changing shape of the molecules in solution, and the shape assumed by the molecule depends in turn on its intensive flexibility as well as on its chain length, the nature of the solvent, the temperature of the solution, and the concentration. If in comparing different polymer-solvent systems these other things can be made "equal", it should be possible by determinations of intrinsic viscosity at various temperatures and in different solvents to obtain a measure of the relative flexibility of polymer molecules.

Acknowledgments

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THE EFFECT OF X- AND γ -RAYS ON AQUEOUS SOLUTIONS OF SODIUM THYMONUCLEATE¹

BY G. C. BUTLER²

Abstract

Aqueous solutions of sodium thymonucleate have been irradiated with X- and γ -rays and the effects have been assessed by measuring the changes in the viscosity of the solutions. The relations of change produced to total dosage, to the presence of protective agents, to concentration of solution, and to dosage rate indicate that the radiations act through the mediation of activated water. The relation of change produced to the state of the nucleate before irradiation suggests that irradiation initiates, by chemical action, a process that may thereafter proceed spontaneously.

Introduction

More information is needed about the chemical processes involved in the biological action of ionizing radiations; see Lea (7) and Allsopp and Catcheside (1). Since thymus type nucleic acid is a prominent constituent of chromosomes (11), although its precise biological function is not understood, it was hoped that an investigation of the chemical effects of ionizing radiations on this material might yield results of biological interest. It was also felt that the irradiation of nucleic acid might prove to be a useful tool for studying the macromolecular structure of the highly polymerized material. From a chemical point of view the use of radiations in such studies offers one unique advantage; it brings about changes in aqueous solutions without introducing any persistent foreign chemical.

An investigation with the above objects in view was conceived as falling into three parts, (a) a quantitative study of the changes produced by irradiation, (b) the identification of some of the processes involved, and (c) the study of the chemical alterations in the irradiated material. The present communication deals with phase (a) of the investigation.

A striking characteristic of solutions of highly polymerized sodium thymonucleate is their high degree of structural viscosity, and this is the property that was chosen for study in the present work. This property has the advantage that it readily lends itself to measurement with simple apparatus, and many workers have used intrinsic viscosity measurements as a measure of relative molecular weights. With such large and asymmetric particles as those formed by sodium thymonucleate in solution, measurements of intrinsic viscosity are not possible because a concentration-viscosity plot does not give a straight line and the slope cannot be estimated accurately at very low concentrations. Furthermore the apparent viscosity is markedly dependent on the rate of shear of the solution in the viscometer, and this effect is most noticeable with the low rates of shear obtained in the usual viscometers.

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For these two reasons it is impossible to obtain absolute viscosity values that may be related to particle size and shape. Consequently, most of the results of the viscosity measurements in the present work are expressed simply as the parameters of an equation relating rate of shear to shear stress. These parameters may be related to viscosity or structural viscosity under specified conditions.

It has been found that for a given polymer in solution the viscosity of the solution increased with the degree of polymerization of the solute. For this reason most workers assume that a reduction in the structural viscosity of solutions of a given sample of sodium nucleate is to be associated with a decreased axial ratio and a decreased molecular weight. In spite of the fact that the exact relation between these two properties and viscosity is not known it was felt that viscosity measurements were significant in a relative sense and could be used to obtain information about the nature of the radiation effect.

The criteria that have been formalized by Lea (7) have been used to determine if the action of the radiations is due to ionization within the affected material (direct action) or is brought about by the intervention of activated water molecules (indirect action). In order to identify such an indirect effect it is necessary to show that, (1) the shape of the curve relating yield to total dose is linear, (2) the presence of some foreign substance in the solution along with the material being irradiated results in a reduction of the yield due to irradiation, (3) above a certain critical concentration the absolute amount of change in the solute is independent of solute concentration. In the present work these three conditions have been met and in addition the results of an experiment with the effect of dosage rate on yield may be explained by an indirect action.

A number of samples of sodium thymonucleate with widely differing viscosity properties were subjected, in solution, to irradiation with radium γ -rays for identical periods and with identical dosage rates. It was found that the amount of change produced was directly proportional to the initial viscosity of the sample irradiated, and these results have led to some speculation about the nature of the depolymerization process.

During the course of this work two other reports on the same subject were made by Sparrow and Rosenfeld (13) and by Taylor, Greenstein, and Hollaender (14), respectively.

Materials and Methods

Viscosity Measurements

These measurements, except those in one experiment, were made with a modified Ubbelohde (15) suspended level type of viscometer having a capillary of radius 0.048 cm. and length 7.44 cm. The reservoir of the viscometer was a 5 ml. graduated pipette from which the solution was allowed to flow under the influence of gravity through the capillary, and the times of flow for successive 0.5 ml. portions were measured. All viscosity measurements were

made at $25 \pm 0.1^\circ \text{C}$. From the flow rates so obtained, the Hagenbach-Poiseuille formula, and the dimensions of the viscometer, rates of shear were calculated for shear stresses that varied from 32.6 to 87.0 dynes per cm^2 in the case of the above instrument.

For a 0.5% solution of sodium thymonucleate in the viscometer just described it was found that the rate of shear did not vary linearly with the shear stress. When the logarithms of the data are plotted as in Fig. 1 it

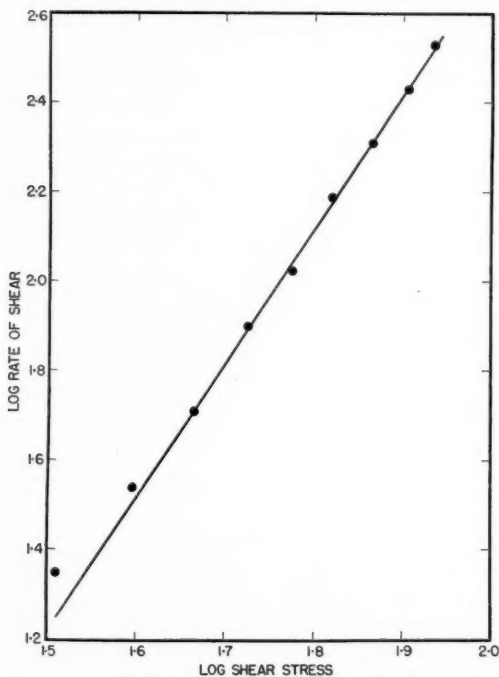


FIG. 1. Typical plot of log rate of shear against log shear stress derived from flow rate measurements with the Ubbelohde viscometer on a 0.5% solution of sodium thymonucleate.

becomes evident that the rate of flow of the solution through a capillary can be expressed by the equation $R = KS^m$, where R is the rate of shear, S is the shear stress, m is the slope of the straight line, and K is a constant. With the same apparatus it was found that this relation held for shear stresses as high as 170; however, it can be seen that there is some deviation from the linear relation at low rates of shear and this is probably due to factors that are not understood in the case of solutions with such a degree of anomalous viscosity.

The above equation has been found by Farrow, Lowe, and Neale (3), Wo. Ostwald (12), and others to apply to a variety of colloidal solutions, and it has been found convenient in the present work to summarize the results

obtained in viscosity measurements, in terms of values for K and m . In the case of solutions prepared from the same sample of "dry" nucleate at different times the variations in m were $\pm 5\%$; for different samples of the same solution of nucleate, determinations of m were reproducible to within $\pm 1\%$.

It was also found (Fig. 2) that the values of $\log K$ and m were proportional to the concentration of sodium thymonucleate in solution.

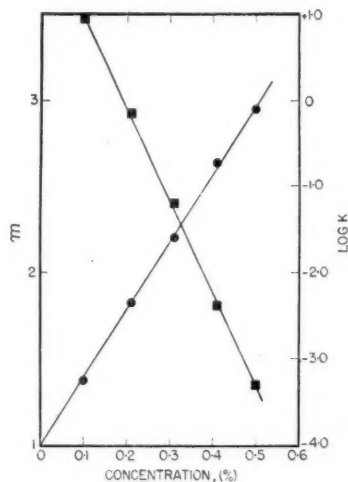


FIG. 2. ●—● change of m with change of concentration of sodium thymonucleate solution (left-hand ordinate scale).
 ■—■ change of $\log K$ with change of concentration of sodium thymonucleate solution (right-hand ordinate scale).

The investigation of the protective action of glucose, methanol, hydrogen peroxide, and irradiated nucleate was made using an Ostwald type of pipette (A.S.T.M. No. 100) containing 5.0 ml. of solution. This pipette was in a water bath at $25 \pm 0.1^\circ \text{C}$. during all measurements.

Sodium Thymonucleate and Thymonucleoprotein

As a result of considerable experience in the preparation of thymus nucleate it was found that the procedure of Mirsky and Pollister (10) consistently yielded more viscous preparations than did the method of Hammarsten (6). Consequently all the samples used in the present investigation were prepared by the former method, with some of the modifications suggested by McCarty and Avery (8) and Gulland, Jordan, and Threlfall (5).

Although great care was taken to work at low temperatures there was a great variability in the viscosity property of solutions of the final products. This is illustrated by the data in Table I, which are a representative selection from a much larger number of batches. Sodium citrate (0.01 M) was present in all solutions used in the preparation of Batches XI and XII and this seemed

TABLE I
 VISCOSITY PROPERTIES OF DIFFERENT BATCHES OF SODIUM THYMONUCLEATE
 (ALL MEASURED IN 0.5% SOLUTIONS)

Batch No.	m	$\log K$
<i>I c</i>	3.79	-5.18
<i>V</i>	2.18	-1.66
<i>VI</i>	1.54	-0.22
<i>VIII</i>	3.03	-3.42
<i>IX</i>	1.25	0.89
<i>X a</i>	2.92	-3.08
<i>X b</i>	2.70	-2.22
<i>X d</i>	1.49	0.04
<i>XI</i>	4.50	-6.36
<i>XII b</i>	3.26	-3.73

to yield a more viscous product. For all these batches the N : P ratios lay between 1.60 and 1.65 and the ratios of the extinction coefficient for aqueous solutions at 260 $m\mu$ to that at 230 $m\mu$ lay between 2.57 and 2.66. In spite of seven or eight emulsifications with chloroform until no more denatured protein appeared at the chloroform-water interface, all samples gave a faintly positive biuret test.

The thymonucleoprotein was prepared by the method of Mirsky and Pollister (10) and the final solution in 1 *M* sodium chloride was clarified by filtration through a loose mat of washed asbestos.

Irradiations

(a) With 200 kv. X-rays

The source of radiation in these experiments was a General Electric OX-220 X-ray machine operated at 200 kvp. and a tube current of 10 ma. The unfiltered beam was directed downward onto the sample, which was contained in a glass-stoppered weighing bottle inside a copper box with an aluminum lid 1 mm. thick; the interior of the box was kept at $25 \pm 1^\circ \text{C}$. Dosage measurements were made with a 100 r. Victorsen condenser chamber substituted as closely as possible in the position occupied by the sample. In this series of experiments the dosage rate was always 200 ± 10 r. per min.

(b) With 2 Mev. X-rays

For carrying out the irradiations at varying dosage rates the source of X-rays was a General Electric 2 Mev. X-ray machine that was operated at 2 Mev., and the tube current varied between 10 and 1000 μa . while the sample was kept at a fixed distance from the target. The X-ray beam was directed horizontally into a closed lead cylinder with walls 4 in. thick and an end 12 in. thick. To avoid uncertainties in dosage rate during the "working up" time of the machine, it was arranged to lower the sample into the beam, from a shielded position outside the cylinder, after the desired tube current had been established.

Solutions of sodium thymonucleate were irradiated in a closed polystyrene cell at temperatures ranging from 25° to 29° C. The cell was an exact replica of the ionization chamber used for dosage measurements, with the exception of some minor differences pertaining to the electrical measuring circuit. The dosage rates were thus measured by direct substitution.

The cylindrical ionization chamber was constructed of polystyrene with an adequate wall thickness to build up electronic equilibrium, and the wall was made conducting with Aquadag. The collecting electrode was a solid concentric cylinder of aluminum insulated with polystyrene and protected with a guard ring. The lower ends of both the collector and chamber were hemispherical and the effective chamber volume was 11.10 cc. The ionization current was measured by a null method, using a known high resistance; the voltage across the resistor was opposed by a measured e.m.f., the null point being determined with a sensitive electrometer. In all dosage rate measurements, care was taken to achieve saturation. From the current in the chamber and from careful measurements of chamber volume the dosage rates in roentgens were determined; these are given in Table V.

(c) *With radium γ -rays*

Two 1 gm. radium sources in monel metal containers having walls 1 mm. thick were in turn enclosed in a cylindrical aluminum holder; these provided the gamma radiation for these experiments. The solution to be irradiated was contained in a thin layer between two concentric cylinders of polystyrene, the inner cylinder being hollow so that the radium source could be inserted therein. All irradiations were carried out within a protecting lead shield at a temperature of $25 \pm 1^\circ$ C. The dosage rate was again measured by direct substitution; for the measurements the inner wall of the outer cylinder was coated with Aquadag and it served as the high voltage electrode while the outer wall of the inner cylinder was similarly treated and it served as the collector. The effective chamber volume was 9.79 cc. The ionization current in the chamber was measured by a null method as described above.

(d) *With Co^{60} γ -rays*

In one series of experiments approximately 2 c of radioactive cobalt (Co^{60}) was substituted for the radium source in the above arrangement.

Unless described otherwise the procedure for the irradiation of solutions of sodium thymonucleate was as follows. On the day of the experiment the dry sodium nucleate was weighed out and to it was added distilled water to give a solution of the desired concentration. The nucleate was dissolved by gentle agitation and when solution was complete (usually in one hour) the solution was divided into two portions, one being placed in the vessel for irradiation, the other being placed in a similar vessel to serve as a control. The control solution was stored, during the time of irradiation, at the same temperature as that at which the irradiation was carried out. Just before the end of the

irradiation the viscosity measurements were carried out on the control solution and immediately after the cessation of irradiation on the irradiated solution.

Taylor *et al.* (14) have reported that there was a gradual spontaneous decrease in the viscosity of their sodium thymonucleate solutions after cessation of irradiation and this phenomenon was also encountered in the present work. This makes it a difficult, and arbitrary, matter to select a time after irradiation for comparing viscosities. It was found that this behavior varied considerably from batch to batch of nucleate and that the fall, though slight, was quite rapid for a few minutes after irradiation but thereafter became much more gradual. The procedure adopted in all the present work was to carry out viscosity measurements at five-minute intervals until three consecutive sets of readings checked to within 1% and to take the mean of these as the recorded value. This relatively steady state was usually reached within 45 min.

Results

(a) Relation of Yield to Total Dose

The results of three separate experiments are plotted in Fig. 3. The data for each curve were obtained by irradiating samples of the same batch of

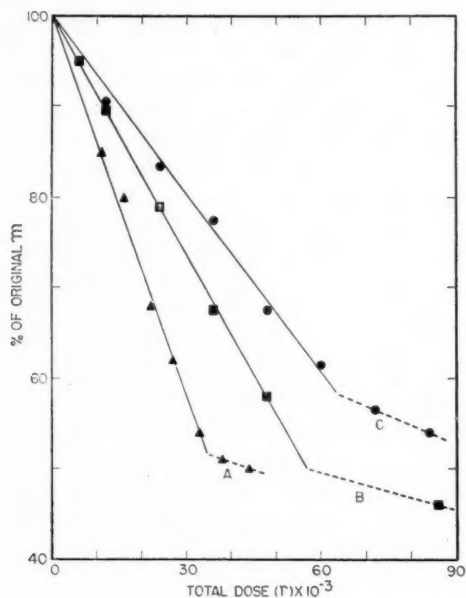


FIG. 3. Relation between viscosity and total dose of radiation for 0.5% solutions of sodium thymonucleate.

- A, \blacktriangle — \blacktriangle STN Batch VIII, radium γ -rays.
 B, \blacksquare — \blacksquare STN Batch VIII, 200 kv. X-rays.
 C, \bullet — \bullet STN Batch X a, 200 kv. X-rays.

sodium thymonucleate at the same intensity for varying lengths of time and measuring the difference between the viscosity properties of the control and irradiated samples.

The curves show that, when the decrease in m is used to measure the change effected, there is an approximately linear relation between dose and yield over a wide range of total dosage. There was a similar relation between $\log \bar{K}$ and the total dose received. In each experiment the value of m at which the discontinuity in the curve appeared was approximately 1.5.

Another experiment was carried out under different conditions using a more dilute solution of sodium thymonucleate (0.05%), which had sodium chloride present in a concentration of 0.02 M . Samples of this stock solution were irradiated for various periods with Co^{60} γ -rays and the specific viscosities of the solutions were obtained from measurements with the Ostwald viscometer. The results of this experiment are plotted in Fig. 4.

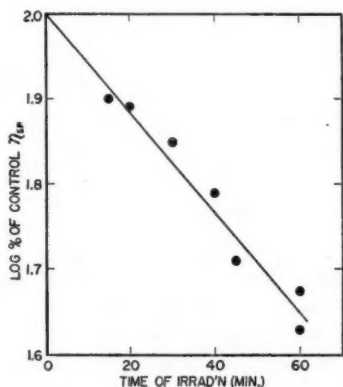


FIG. 4. The relation between viscosity and total dose of radiation. Solution (0.05%) of thymonucleate, Batch XXI in 0.02 M sodium chloride irradiated with Co^{60} γ -rays at 1.94 r . per sec.

The residual specific viscosity is close to being a linear function of total dose but the experimental values fit a straight line slightly better when they are plotted as in Fig. 4.

It therefore appears that the shape of the yield-dosage curve may depend on the experimental conditions used for obtaining it. The difference in the two results might be due to the strong gel formation in more concentrated solutions of thymonucleate in distilled water.

There can be two reasons for an exponential relation between yield and total dose; (a) the radiations act directly on the solute, and (b) the radiations produce activated water, which in turn reacts with the solute both before and after it has been altered by irradiation (2). The results of subsequent experiments showed that it was the second of these two factors that was responsible for any deviation from linearity of the yield-dosage curve.

(b) Effect of Protective Agents

Instead of measuring the protective effect of a foreign protein the nucleate was irradiated in its natural combination with histone. The solution that was irradiated was a 1% solution of thymonucleoprotein dissolved in 1 *M* sodium chloride; this solution had: N, 0.91 mgm. per ml. and P, 0.38 mgm. per ml. This phosphorus content was the same (within 5%) as those of the 0.5% solutions of sodium nucleate solutions used in other experiments. One hundred milliliters of the solution was prepared and stored at 5° C.; on each of three successive days two 8 ml. samples were removed to serve as the control and irradiated solutions, respectively. The results are summarized in Table II.

TABLE II

THE EFFECT OF PROTECTIVE AGENTS. IRRADIATION OF 1% SOLUTION OF THYMUS NUCLEOPROTEIN IN 1 *M* SODIUM CHLORIDE WITH 200 KV. X-RAYS, 200 R. PER MIN.

Time of irradiation, hr.	<i>m</i>			log <i>K</i>		
	Control	Irradiated	Δm	Control	Irradiated	$\Delta \log K$
1	1.89	1.85	-0.04	-1.01	-0.92	0.08
2	1.87	1.78	-0.09	-0.87	-0.71	0.16
4	1.88	1.69	-0.19	-0.98	-0.50	0.48

It can be seen that the effect of the irradiation on the nucleoprotein solution is much less than that on a sodium nucleate solution. There are two possible explanations for this result. One is that the nucleic acid component, even though it was chemically altered by the irradiation, might be held in its original physical form by union with the histone. The other is that a large proportion of the activated water molecules reacted with the histone instead of with the nucleic acid. Although it was felt that the former explanation was unlikely it seemed clear that the effect of protective agents other than protein should be studied. Accordingly the protective actions of glucose, methanol, hydrogen peroxide, and irradiated sodium thymonucleate were measured.

The irradiated thymonucleate referred to was prepared as follows: Ten milliliters of a 0.5% solution of sodium thymonucleate, Batch XXI, 0.02 *M* with respect to sodium chloride was irradiated for 16 hr. with Co⁶⁰ γ -rays at 1.94 r. per sec. When a sample of this solution was diluted tenfold with 0.02 *M* sodium chloride solution it was found that its specific viscosity had been reduced from 0.94 to 0.08. This 0.5% solution, after irradiation, was used in the protective experiments.

The nucleate was dissolved in water, sodium chloride and a solution of the protective agent were added, and the volume of the solution was adjusted by the addition of water to give a solution containing 0.05% nucleate and 0.02 *M* sodium chloride. A number of such solutions were prepared each containing a different amount of the protective agent under study.

Samples of a solution containing any one concentration of protective agent were irradiated for various times with γ -rays from Co^{60} at a dosage rate of 1.94 r. per sec. The times of irradiation were chosen so that the longest irradiation reduced the specific viscosity of the solution more than 50%. Then, by plotting the percentage residual specific viscosity against time of irradiation, a curve similar to that in Fig. 4 was obtained. From such a curve the time of irradiation necessary to reduce the specific viscosity by 50% ("half-dose", D_1) could be obtained by interpolation. These "half-doses" for a number of different concentrations of protective agent were plotted against concentration of protective agent. Fig. 5 is the plot obtained for

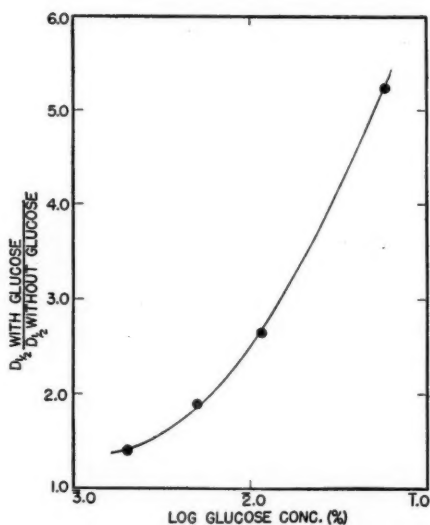


FIG. 5. The effect of glucose on "half-dose". Solution (0.05%) of thymonucleate, Batch XXI in 0.02 M sodium chloride irradiated with Co^{60} γ -rays at 1.94 r. per sec.

glucose. From such plots the concentration of protective agent necessary to double the "half-dose" was read off. These are the concentrations that give 50% protection of the nucleate against the action of activated water and that are recorded in Table III.

Hydrogen peroxide had no protective or enhancing action in a concentration of 0.3%.

Results such as these make it possible to compare the relative affinities of activated water for different substances in solution.

(c) Effect of Concentration of the Solute

Sodium thymonucleate, Batch V, in 0.25, 0.50, and 1.0% aqueous solutions was irradiated with 200 kv. X-rays, the total dose being 12,000 r. The results are summarized in Table IV.

TABLE III

THE RELATIVE EFFECTIVENESS OF PROTECTIVE AGENTS. SOLUTION OF BATCH *XXI* SODIUM THYMONUCLEATE (0.05%) IN 0.02 *M* SODIUM CHLORIDE IRRADIATED WITH Co^{60} γ -RAYS AT 1.94 R. PER SEC.

Protective agent	Concentration for 50% reduction in yield
Glucose	0.31 mM.
Methanol	0.69 mM.
Irradiated sodium thymonucleate	0.042%

TABLE IV

THE EFFECT OF CONCENTRATION ON YIELD. BATCH *V* SODIUM THYMONUCLEATE IRRADIATED WITH 200 KV. X-RAYS FOR ONE HOUR AT 200 R. PER MIN.

Concentration at irradiation, %	Conc'n at viscometry, %	<i>m</i>			Yield*
		Control	Irradiated	Δm	
0.25	0.25	1.81	1.68	-0.13	7.2
0.50	0.50	2.23	2.07	-0.16	7.2
1.0	0.50	2.24	2.14	-0.10	8.9

$$* \text{Yield} = \% \text{ reduction in } m \times \frac{\text{conc'n. at irradiation}}{\text{conc'n. at viscometry}}$$

It was not feasible to use a greater range of concentration because of the inconvenience of measuring the viscosity property of solutions that were either too concentrated or too dilute. But within the range of concentration studied (fourfold) it is apparent that for a given dose the absolute yield did not vary greatly. In order to study the effect of solute concentration further, a sample of Batch *VIII* was irradiated in the "dry" state. Two 50 mgm. samples of this sodium nucleate were weighed and one sample was placed in each of two 10 ml. volumetric flasks. One sample received 24,000 r. of 200 kv. X-rays in two hours after which 8 ml. of water was added to each flask and the nucleate dissolved by gentle agitation. The volumes were adjusted to 10 ml. with water, the solutions thoroughly mixed, and the viscosity behavior of each solution was measured as usual. The values obtained were as follows:

	<i>m</i>	log <i>K</i>
Control	3.26	-4.95
Irradiated	3.21	-4.85

The effect of 24,000 r. of X-rays was therefore negligible in the absence of water. The results of these two experiments provide further evidence that the main effect of X-rays on nucleate solutions is mediated by the water in which the nucleate is dissolved.

(d) *Effect of Dosage Rate*

Samples of sodium thymonucleate, Batch *X b*, in 0.5% solutions were irradiated with 20,000 r. of 2 Mev. X-rays at rates varying between 1.6 and 124 r. per sec. The viscosity changes resulting from the irradiations are summarized in Table V.

TABLE V

EFFECT OF DOSAGE RATE ON YIELD. IRRADIATION OF SODIUM THYMONUCLEATE, BATCH *X b*, IN 0.5% SOLUTION WITH 2 MEV. X-RAYS, TOTAL DOSE 20,000 R.

Current in X-ray tube, ma.	Dosage rate, r./sec.	Control solution		Irradiated solution		Δm
		<i>m</i>	log <i>K</i>	<i>m</i>	log <i>K</i>	
0.01	1.6	2.68	-2.70	2.21	1.65	-0.47
"	"	2.62	-2.62	2.11	-1.43	-0.51
0.03	4.5	2.65	-2.63	2.21	-1.63	-0.44
"	"	2.66	-2.62	2.24	-1.71	-0.42
0.10	12.9	2.72	-2.78	2.35	-1.96	-0.37
"	"	2.70	-2.70	2.33	-1.89	-0.37
1.0	124	2.69	-2.69	2.29	-1.87	-0.40
"	"	2.65	-2.65	2.26	-1.80	-0.39

During these experiments there arose two technical difficulties that should be mentioned. Firstly, it was very difficult to control the tube current at a constant value of 0.01 ma. and this difficulty probably explains the poor agreement between the two values of Δm recorded for the first experiment. Secondly, in order to carry out the irradiation at 124 r. per sec. it usually required about 30 min. to develop a current of 1 ma. in the X-ray tube, and during this period the sample, in its position just outside the lead cylinder enclosing the X-ray beam, probably received an appreciable irradiation before it was placed in the beam. Therefore in the last experiment the total dose was probably greater than 20,000 r. and this is the only reason that can be given for the unexpectedly high yield obtained with the highest dosage rate.

From the results of the first three experiments it can be seen that there is a small and regular decline in yield as the dosage rate is increased from 1.6 to 12.9 r. per sec. This finding can be most readily explained as follows. Suppose that on irradiation water molecules are activated by being converted into two kinds of reactive fragments, *A* and *B*, and that *A* reacts with the dissolved nucleate to alter its structure or with *B* to re-form water. Nucleate and *B* will therefore compete for the available *A*. Under the conditions of the experiment just described increasing the dosage rate will increase the concentration of *B* while the concentration of nucleate remains constant so that a greater proportion of *A* will be reacting with *B* at high dosage rates than at low ones. This could account for the reduction in yield with increasing dosage rates but since the reduction in yield is slight the reaction of *A* with *B* may be regarded as small in comparison with the reaction of *A* with nucleate.

(e) *Effect of Condition of the Nucleate before Irradiation*

Eight different samples of sodium thymonucleate in 0.5% solutions received identical irradiations with radium γ -rays; the results are presented in Table VI. With the exception of the two samples with the lowest viscosities

TABLE VI

VARIATION OF YIELD WITH DIFFERENT SAMPLES. SOLUTIONS (0.5%) OF SODIUM THYMONUCLEATE IRRADIATED FOR TWO HOURS WITH RADIUM γ -RAYS AT 91 R. PER MIN.

Batch No.	m (for control sol'n.)	$-\Delta m$ (due to irradiation.)	$\frac{-\Delta m}{m-1}$
<i>I c</i>	3.79	0.73	0.26
<i>V</i>	2.18	0.31	0.26
<i>VI</i>	1.54	0.10	0.19
<i>VIII</i>	3.03	0.45	0.22
<i>X a</i>	2.92	0.45	0.24
<i>X d</i>	1.49	0.04	0.08
<i>XI</i>	4.50	1.00	0.29
<i>XII b</i>	3.26	0.58	0.26

(*VI* and *X d*) the values in the last column are fairly constant, showing that the yield for a given amount of irradiation is proportional to the degree of anomaly of the viscosity of the solution that is irradiated.

It is interesting to note that the initial values of m for 0.5% solutions of samples *VI* and *X d* are near 1.5 where it was noted the discontinuities occurred in the curves of Fig. 3.

The results obtained with the remaining six samples can most easily be explained by assuming that the initial value of $m - 1$ is directly proportional to the degree of polymerization or that samples with the highest values of $m - 1$ show the greatest departure from some unpolymerized state. It would then be possible to obtain the results recorded in Table VI if the depolymerization proceeded in two stages as follows. (a) A certain number of chemical events take place in the macromolecule and initiate the process of depolymerization. These chemical events are probably the reaction of activated water with bonds in the macromolecule and they could be the same for all samples. (b) Each macromolecule in which this initial process has happened then depolymerizes spontaneously from its initial state to an unpolymerized state.

If process (b) were slow then the above scheme would provide an explanation for the slow spontaneous decrease that takes place in the viscosity of irradiated solutions after cessation of the irradiation.

(f) *Quantitative Considerations*

It is of some interest to estimate the number of active radicals that must react with unit weight of thymonucleate to produce, say, a 50% reduction in the specific viscosity of a solution. From Fig. 4 it can be seen that 5,900 r.

of γ -rays will reduce the specific viscosity of a 0.05% solution of batch *XXI* thymonucleate by 50%. Miller (9) has shown that 1000 r. of γ -rays will bring about the oxidation of 19.5 μ M. per liter of ferrous ions in an air-saturated solution. Dewhurst and Krenz (private communication) have found that, in an oxygen-free solution, 5 μ M. per liter of ferrous ion is oxidized per 1000 r. The fourfold greater yield in the presence of oxygen is to be explained by oxidizing agents formed on the interaction of activated water and oxygen. It may thus be assumed that 1000 r. of γ -rays produces 5 μ M. of active radicals of one kind per liter.

Irradiation of thymonucleate solutions gave the same reduction in viscosity in the presence and in the absence of oxygen; so it follows that 59 μ M. of active radicals react with one gram of sodium thymonucleate to reduce the specific viscosity by 50%. Similar calculations based on similar data for Batch *XIII* thymonucleate give a corresponding value of 81 μ M. per gram. Assuming that one gram of thymonucleate can react with twice these amounts of active radicals, one obtains a value of 8500 for the effective molecular weight of Batch *XXI* and 6200 for Batch *XIII*.

These values for the number of active radicals reacting may be in error because it is assumed that one active radical of one kind, produced by the action of the γ -rays on water, (i) oxidizes one ferrous ion and, (ii) reacts productively with thymonucleate. It is difficult to assess the validity of the first assumption which leads to a minimum estimate of the number of active radicals produced by the radiations. The second assumption is a maximum estimate; it must be too high by at least 30% because it was shown in Section (b) that irradiated nucleate had an affinity for activated water equal to that of the unirradiated material.

In spite of their uncertainty, calculations of the sort just made seem to be more informative than estimates of ionic yield for which one has to assume an effective molecular weight. Any estimate of ionic yield also involves the assumption that one ion pair is produced in 1 ml. of water by the absorption of 32 e.v. of energy from the radiations; this number has never been settled by experiment. Using a molecular weight value of one million (that obtained in some sedimentation studies) for sodium thymonucleate, one obtains an ionic yield of 0.02 for the viscosity reduction of Batch *XXI* by irradiation.

Discussion

The conclusions to be drawn from each experiment have been given in the previous section. It remains to consider their interpretation.

A correlation of the results is made difficult by the fact that the material under study has an unknown constitution and an uncertain molecular weight. It seems most probable that the reduction in viscosity by irradiation results from an alteration of inter- or intra-molecular linkages in the macromolecules but there is no evidence indicating the nature of these linkages. As a result

of their study of the reduction in the viscosity of sodium thymonucleate solutions on treatment with acid or alkali, Gulland and Jordan (4) suggested that the linkages affected were hydrogen bonds between amino and hydroxyl groups. Such bondings, if they exist, might be the site of action of the ionizing radiations, but this or any other theory of the site of action is purely speculative at present.

Whatever is the point of attack of the ionizing radiations on the nucleate, there seems very little doubt, as a result of this investigation, that the attack is mediated by activated water molecules and that direct ionization in the nucleate molecules has by comparison a negligible effect.

It is doubtful if the results obtained so far in this work have any direct implications for biology. There is no way of knowing what is the native physical state of the nucleic acid in the cell nucleus. It seems to be implied in all communications dealing with the isolation of thymus type nucleate that the greater the degree of anomalous viscosity of the isolated product the less degraded it is and therefore the more does it resemble its original state in the living cell. One must always consider the possibility, however, that the high degree of structural viscosity may be brought about by the isolation process.

The doses required to produce a measurable effect on the sodium nucleate are far greater than those required to bring about striking changes in the nuclei of living cells, but this is not surprising in view of the very large number of individual chemical events that would be necessary to give rise to a measurable chemical or physical change in the material. From a strictly biological point of view it would be much more interesting to irradiate a nucleic acid such as the pneumococcus-transforming principle of McCarty and Avery (8), which has a biological action in very small amounts. It is possible that with such a material a measurable alteration could be obtained with much smaller doses of radiation.

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MEMBRANE EQUILIBRIA, THE GIBBS-DALTON LAW AND THE ENTROPY OF MIXING¹

BY E. A. FLOOD AND G. C. BENSON

Abstract

When two pure fluids whose pressures are p_{1e} and p_{2e} , respectively, are separated by means of semipermeable membranes from a mixture of these fluids, and under equilibrium conditions the pressure of the mixture is P , then the net pressures sustained by the diaphragms are $P - p_{1e}$ and $P - p_{2e}$ respectively. The assumption that these net pressures are p_{2e} and p_{1e} , respectively, is equivalent to assuming the Gibbs-Dalton law, namely $p_{1e} + p_{2e} = P$. It is shown that the Gibbs-Dalton law when applied to fluids that are not ideal gases leads to consequences which are entirely contrary to experience and that as applied to ideal gases it has neither an experimental nor theoretical thermodynamic basis. It is shown that the Gibbs-Dalton law is only a special case of Dalton's law and that the classical thermodynamic paradox in the entropy of mixing of ideal gases is based on the erroneous assumption that the Gibbs-Dalton law necessarily holds when Dalton's law holds. It is shown that when two ideal gases obey Dalton's law of mixing, it is thermodynamically quite possible for the equilibrium pressure of one pure gas to be increased while that of the other is decreased, as well as the more familiar case of "chemical reaction", where the equilibrium pressures of both are decreased. It is shown that there is no purely thermodynamic requirement that different kinds of molecules in mixtures of ideal gases shall have the same mean translatory kinetic energy. The ideas underlying membrane equilibria are discussed in some detail. Some general condition equations which must be met are given, together with a few explicit solutions of these equations for special simple cases.

I. Introduction

All fluids which are appreciably different from one another react with one another to a greater or lesser degree, when allowed to intermix. The extent to which such reaction occurs often depends on highly specific subtle factors inherent in the natures of the fluids. Usually such specific factors are not included in the statistical models of the fluids concerned. The omission of these factors introduces uncertainties in the theoretical treatment of the mixing of the statistical models and these uncertainties lead, in turn, to uncertainties in the statistics of mixtures of even the simplest of statistical models of thermodynamic fluids. Before considering statistical or mechanical details of such models it is necessary to ensure that the model fluid mixture fulfill two requirements. The first and absolutely essential requirement is that the mixing behavior of the fluid models be thermodynamically possible, otherwise the terms "temperature", "energy", etc., as applied to the models are practically meaningless. The second requirement is that the behavior of the model be physically reasonable, in the sense that it should not be in conflict with well established experience, and should, if possible, be free from paradoxes.

This paper presents some thermodynamic and physical considerations concerning the mixing of fluids, special emphasis being placed on the mixing of

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ideal gases. While this subject has been discussed at length in the scientific literature, there are one or two points that appear to have been overlooked, to which attention is invited in the following pages.*

Membrane Equilibrium, the Entropy of Mixing, and the Gibbs-Dalton Law

"Membrane equilibrium", "the entropy of mixing," and the "Gibbs-Dalton law" are three commonly used expressions denoting ideas which are closely associated with one another and with the thermodynamics of fluid mixtures.

If we imagine a given fluid to be separated by means of a semipermeable membrane from a mixture of the given fluid and other fluids and we suppose that the membrane allows the given fluid to pass through it quite readily but prevents passage of the other fluids, and we now suppose that the other fluids in the mixture literally *act as a vacuum* to the given fluid, we must conclude that the concentration of the given fluid will be the same on either side of the membrane under equilibrium conditions. If, however, there is any sort of thermodynamic interaction between the given fluid and the other fluids in the mixture, then under equilibrium conditions there will be, in general, a difference between the concentrations of the given fluid on either side of the membrane. This difference in concentrations or molar densities will constitute a measure of the degree of thermodynamic interaction of the given fluid with the others in the mixture.

It might at first be thought that a fluid could never act as a vacuum to another fluid, but when we consider the possibilities of expanding different, very dilute gases into one another through semipermeable membranes, one is inclined to think that there should be a very general class of cases where volumes of different fluids act as empty spaces to one another, in spite of the paradoxical fact that volumes of the same fluid cannot act as empty spaces to one another under any finite circumstances, however dilute they may be, however closely they may approach a vacuum.

The paradoxical nature of these ideas has been set forth by many writers, especially by Gibbs in connection with the "entropy of mixing" and leads to the well known Gibbs paradox. We shall see, however, that the paradox is, perhaps, more imaginary than real and owes its origin to unwarranted assumptions concerning equilibrium pressures across semipermeable membranes.

The "entropy of mixing" of fluids, as the expression is generally used, refers to the entropy change, if any, which accompanies the removal of a partition separating two fluids which are under the same pressure and which have the same temperature. It may be taken as axiomatic that if the fluids have the same

* While the literature dealing with this classical subject is so voluminous that it is impractical to provide anything like a complete bibliography, we would like to acknowledge a few of the sources to which we are most indebted.

These are: *The Collected Work of Willard Gibbs and Donnan and Haas's valuable commentary thereon; the series of scientific papers on this subject by Louis Gillespie; the papers of Beattie and of Guggenheim on the thermodynamics of gas mixtures; the standard thermodynamic textbooks, notably those of Lewis, Epstein, and Guggenheim.*

temperature and pressure, and surface and external forces are negligible, no entropy change will occur on removal of the partition, if the fluids are identical. However, if the fluids are not identical it is practically impossible to predict from a priori considerations whether a finite entropy change will occur or not. Neither very close similarity nor very great differences in the thermodynamic and physical properties will enable one to make any accurate a priori calculations of the entropy of mixing. If the two fluids are not identical and are miscible in the sense that a uniform distribution of material throughout the whole volume is rapidly attained after removal of the partition, usually an entropy change will occur. Usually there will also be a change in the energy of the system and in the value of (PV) , and hence, a change in the values of the thermodynamic functions H , A , and F .^{*} If a chemical reaction occurs and $(-\Delta F)$ is large, usually $(-\Delta E)$ is also large, but $(-\Delta F)$ may be large for reactions where (ΔE) and $\Delta(PV)$ are both practically zero. In such cases the large value of $(-\Delta F)$ for the reaction is due to a large "entropy of mixing" or "entropy of reaction". In cases where (ΔE) and $\Delta(PV)$ are zero and ΔS is comparatively small it might be very difficult to distinguish between "reaction", "solution", and mere "mixing". If we consider that the mere fact that the fluids are different necessarily implies a minimum entropy of mixing, considerably greater than zero, we might find ourselves in difficulties in cases where the entropy change of the process was actually negative, as it is in some reactions. It would be more logical to assume that when no reaction occurs ΔE , ΔH , ΔP , ΔA , ΔF , and ΔS are all zero, as would be the case were the fluids identical. Thus we might be inclined to regard the classical entropy of mixing as an entropy of reaction. When looked at from this point of view, in cases where ΔE and $\Delta(PV)$ are zero, we might expect ΔS to vary from zero through the classical value to very large values depending on the nature of the fluids. Certain types of these reactions might be regarded as chemical reactions, but there will be cases where there is no sharp line of demarcation between "chemical reaction", "solution", and "mixing reaction". This view has at least the merit of logical continuity of ideas.

It seems unreasonable if not altogether illogical to suppose that the "mixing reaction", the "entropy of mixing", in the case of different ideal gases cannot be less than the classical entropy of mixing no matter how small the difference between the fluids might be. Surely, if the two fluids are inert monatomic gases which differ only in the fact that their nuclei have different half lives, say 10^{10} and 10^{11} years, respectively,—surely it would seem that the "entropy of mixing" would not be the same as if the gases had quite different atomic weights, or were altogether different substances? It seems perfectly clear that nothing resembling a semipermeable membrane is capable of separating, *reversibly*, fluids whose only difference lies in the relative stabilities of the nuclei of their atoms. Is there not a fallacy in postulating the existence of a semipermeable membrane such that the equilibrium across it requires

^{*} *Lewis symbols, cf. p. 992 of this paper.*

the concentrations of the inert gas to be the same on either side, irrespective of the presence on one side of a second inert gas which differs from the first gas only in that the nuclei of its atoms are slightly more or less stable? Surely we must know the equilibrium conditions before we postulate the hypothetical membrane? The equilibrium condition has, really, little to do with the semipermeable membrane. The membrane is merely a simple device used for carrying out pencil and paper operations between systems in equilibrium. The paradox that the entropy of mixing of different ideal gases is independent of the nature of the gases and independent of the degree of difference of these gases, is a result of assuming that the pressure of an ideal gas mixture is equal to the sum of the equilibrium pressures of the pure gases when separated by semipermeable membranes from the mixture.* This assumption has been shown by Gibbs to be exactly equivalent thermodynamically to the assumption that each gas is as a vacuum to the other gas, irrespective of the equations of state. The question as to the entropy of mixing of fluids is thus reduced to the practical questions: Do different fluids usually act as empty spaces to one another? Do different ideal gases generally act as a vacuum to one another?

The view that different gases act as a vacuum to one another is attributed to Dalton by Lord Rayleigh (4). It is sometimes thought that Dalton's law, and the notion that gases act as empty spaces to one another, amount to the same thing. But that this is far from the case is obvious when we consider that Dalton's law can be applied in the case of identical ideal gases, while no gas can act as a vacuum to itself or to another portion of the same gas at the same pressure. Gibbs (1, p. 155) summarized the views of his time concerning fluid mixtures as follows "The pressure in a mixture of different gases is equal to the sum of the pressures of the different gases as existing each by itself at the same temperature and with the same value of its potential". Gillespie (2) has called this the Gibbs-Dalton law. Gibbs (1, p. 157) showed that this law was thermodynamically equivalent to the statement that "every gas acts as a vacuum to every other gas" irrespective of the equations of state of the pure gases. Dalton's law is, of course, quite different, namely, "the pressure exerted by a mixture of gases is equal to the sum of the pressures which each gas would exert, if it occupied the space alone". One would not expect this law to be valid for gases which depart appreciably from the perfect gas laws. When confined to perfect gases, Dalton's law is obviously applicable when the gases are identical, whereas the Gibbs-Dalton law is clearly not applicable when the gases are the same. If the Gibbs-Dalton law is generally valid for different ideal gases, the paradox in the entropy of mixing is almost inescapable† but if the Gibbs-Dalton law is not generally valid, the paradox is largely imaginary.

* Cf. footnote on p. 1005.

† Statistical considerations do not in any sense "resolve" the paradox but rather tend to emphasize the self-contradictory nature of the ideas involved. Perhaps the essential question is: Can fluids be identical thermodynamically but differ with respect to nonthermodynamic processes?

In what follows we shall derive the conditions for equilibrium across semi-permeable membranes. We shall examine any general solutions that may be obtained in order to ascertain whether there is any thermodynamic reason why ΔS should have some positive minimum where ΔE and $\Delta(PV)$ are zero for such mixing processes as we have discussed. We shall enquire as to whether the Gibbs-Dalton law has any theoretical validity based on thermodynamic considerations or any physical validity based on experience in the behavior of fluid mixtures generally.

II. Membrane Equilibrium

Thermodynamic Relations

The general condition that changes in the thermodynamic variables of a system shall be reversible is that the heat received by the system during such changes shall be equal to the changes in the energy of the system plus the maximum work (if a finite maximum exist) which the system can do against external forces during such changes. This is virtually Carnot's condition for reversibility of a heat engine and leads to the following well known conditions for thermodynamic equilibrium with respect to various specified variations of a given system:

$$(\delta E)_{S,V,X}, (\delta H)_{S,P,X}, (\delta A)_{T,V,X}, (\delta F)_{T,P,X} \geq 0$$

and,

$$(\delta S)_{E,V,X} \leq 0,$$

where E is the energy, S the entropy, V the volume, T the temperature, P the pressure, $H = E + PV$, $A = E - TS$, $F = H - TS$, and X represents other co-ordinates of the system which are to be held constant during the variation. All of these conditions are equivalent. If the equilibrium is to be a neutral equilibrium with respect to specified changes and if these changes are to be reversible, the above variations must vanish.

When a system consists of various parts which may be transformed into one another, the most generally useful condition of equilibrium with respect to such transformations is, $(\delta F)_{P,T,X} = 0$, since in such transformations we usually assume that the *measurable variables* describing the state of two parts, namely, their temperatures and pressures, are to be held constant while only the various masses change. Hence for a process of transformation of two separate systems into a single system containing the same matter, where the temperatures and pressures of each separate system are to remain constant throughout the process (if such a process exist), the condition that it shall be reversible is that,

$$F_{12} = F_1 + F_2,$$

where the system having the free energy F_{12} contains the same matter as in the separate systems having free energies F_1 and F_2 respectively. While this condition for equilibrium is perhaps the most generally useful, when we

wish to determine an equilibrium with respect to the distribution of matter between constant volumes, the appropriate equilibrium condition is that,

$$(\delta A)_{T,V,X} = 0.$$

This condition for equilibrium is particularly well adapted to the determination of the distribution of a matter between volumes separated by means of semipermeable membranes.

We shall now consider some problems associated with such membranes. Of course, in any case where different bodies are assumed to be in thermal contact, the additional requirement for equilibrium is that the temperatures be the same.

Let the following diagram represent a container divided into three volumes, v_{1e} , v_{2e} and V by partitions (1) and (2).

p_{1e}	P	p_{2e}
n_1	n'_1	
u_1	x	
	n'_2	n_2
	y	u_2
v_{1e}	V	v_{2e}
1	2	

FIG. 1

Let partition (1) be permeable only by the first fluid and let there be n_1 and n'_1 moles of this first fluid contained respectively in v_{1e} and V . Similarly partition (2) is to be permeable only by the second fluid and there are to be n_2 and n'_2 moles respectively in v_{2e} and V . The temperature is to be uniform throughout the three volumes. The pressures exerted against the impermeable walls of each volume are to be p_{1e} , P , and p_{2e} respectively, and are to be uniform throughout each separate volume but are not necessarily equal. We shall assume, that an equilibrium can exist under these circumstances and that the volumes v_{1e} and v_{2e} are so chosen that $n_1 = n'_1$ and $n_2 = n'_2$.

We shall also assume that an equation of state $p = f(n, V, T)$ exists where f is homogeneous and of zero degree in n and V , and that surface effects may be neglected. Then putting $u = \frac{n}{V}$ we may write $f(n, V, T) = g(u, T)$, since g cannot contain n explicitly. Hence $p = p(u, T)$ or at constant temperature $p = p(u)$. We shall find it convenient in what follows to use equations of state of this form.

Referring to Fig. 1, if we let $u_1 = n_1/v_{1e}$, $u_2 = n_2/v_{2e}$, $x = n'_1/V$, and $y = n'_2/V$, evidently relations exist as between u_1 , u_2 and x and y . We enquire as to what may be deduced concerning these relations.

Let us write,

$$u_1 = xf_1(x, y), \quad u_2 = yf_2(x, y). \quad (1)$$

Evidently f_1 and f_2 are subject to the following boundary conditions:

$$f_1(x, y=0) = f_2(x=0, y) = 1. \quad (2)$$

$$xf_1(x, y) = 0, \text{ when } x = 0,$$

$$yf_2(x, y) = 0, \text{ when } y = 0.$$

These are necessary boundary conditions imposed on f_1 and f_2 by the physical nature of the assumed system. However, if the form of the equations of state and the values of the variables permit more than one concentration for a given equilibrium pressure, a variety of relations may exist between u_1 and x even when $y = 0$. If, for example, $p = p_1(u_1) = p_1(u'_1)$, where $u'_1 > u_1$, then assuming only that the pressures and temperatures are uniform, the value of u may have any value from u_1 to u'_1 . Hence a variety of relations between u_1 and x may be consistent with equilibrium. Here we shall assume that u_1 and x refer to the smallest of these values if more than one value is consistent with equilibrium. We shall discuss these boundary conditions more fully after presenting some of the necessary relations which must subsist between the functions f_1 and f_2 .

For a neutral equilibrium to exist between the fluids contained in v_{1e} and v_{2e} and the mixed fluid in V , the temperature of each must be the same and the variation in the Helmholtz free energy, δA , resulting from a change in the distribution of matter as between the constant volumes must vanish. If the distribution of the first fluid between the volumes v_{1e} and V is to be independent of the presence or absence of the system v_{2e} , we may write

$$\delta A = \left(\frac{\partial A_{12}}{\partial n'_1} \right)_{T, V, n_2} \delta n'_1 + \left(\frac{\partial A_1}{\partial n_1} \right)_{T, v_{1e}} \delta n_1 = 0$$

and assuming $n'_1 + n_1 = \text{constant}$, we get,

$$\left(\frac{\partial A_{12}}{\partial n'_1} \right)_{T, V, n_2} = \left(\frac{\partial A_1}{\partial n_1} \right)_{T, v_{1e}}$$

$\left(\frac{\partial A_{12}}{\partial n'_1} \right)_{T, V, n_2}$ is, of course, Gibbs thermodynamic potential of the first component in the mixed system. Representing this quantity by μ'_1 , and letting μ_1 represent the thermodynamic potential of the same component in the system v_{1e} which contains only this substance, the equilibrium condition becomes,

$$\mu'_1 = \mu_1.$$

And similarly,

$$\mu'_2 = \mu_2.$$

To every system having a uniform pressure and temperature throughout, whether homogeneous or not, an equation of the form of Gibbs (97)

$$VdP = SdT + n_1d\mu_1 + n_2d\mu_2 \dots \dots \dots$$

is applicable. Thus for the mixed system we have,

$$VdP = SdT + n'_1d\mu'_1 + n'_2d\mu'_2,$$

and for the first system,

$$v_{1e}dp_{1e} = S_1dT + n_1d\mu_1,$$

while for the second system,

$$v_{2e}dp_{2e} = S_2dT + n_2d\mu_2.$$

Since,

$$\mu'_1 = \mu_1, \quad d\mu'_1 = d\mu_1,$$

$$\mu'_2 = \mu_2, \quad d\mu'_2 = d\mu_2,$$

and,

$$n'_1 = n_1, \quad n'_2 = n_2,$$

and T is constant, after eliminating $n_1d\mu_1$, etc., we get the equivalent fundamental equations,

$$VdP = v_{1e}dp_{1e} + v_{2e}dp_{2e} \quad (3)$$

and,

$$\begin{aligned} dP &= \frac{v_{1e}}{V} dp_{1e} + \frac{v_{2e}}{V} dp_{2e} \\ &= \frac{x}{u_1} dp_{1e} + \frac{y}{u_2} dp_{2e}, \end{aligned} \quad (4)$$

where p_{1e} and p_{2e} and hence u_1 and u_2 may be regarded as independent variables, or we may regard x and y as independent variables*. Choosing x and y as independent variables we get,

$$\begin{aligned} dP &= \frac{x}{u_1} \frac{dp_1}{du_1} \left[\frac{\partial u_1}{\partial x} dx + \frac{\partial u_1}{\partial y} dy \right] + \frac{y}{u_2} \frac{dp_2}{du_2} \left[\frac{\partial u_2}{\partial x} dx + \frac{\partial u_2}{\partial y} dy \right] \\ &= \left[\frac{x}{u_1} \frac{dp_1}{du_1} \frac{\partial u_1}{\partial x} + \frac{y}{u_2} \frac{dp_2}{du_2} \frac{\partial u_2}{\partial x} \right] dx + \left[\frac{x}{u_1} \frac{dp_1}{du_1} \frac{\partial u_1}{\partial y} + \frac{y}{u_2} \frac{dp_2}{du_2} \frac{\partial u_2}{\partial y} \right] dy, \end{aligned}$$

and in order that dP be the derivative of a function,

$$\frac{1}{u_1} \frac{dp_1}{du_1} \frac{\partial u_1}{\partial y} = \frac{1}{u_2} \frac{dp_2}{du_2} \frac{\partial u_2}{\partial x} \quad (5)^\dagger$$

* Footnote on following page.

† It will be noted that under ordinary conditions u_1 , u_2 , $\frac{dp_1}{du_1}$, $\frac{dp_2}{du_2}$ cannot be negative and hence $\frac{\partial u_1}{\partial y}$ and $\frac{\partial u_2}{\partial x}$ or $\frac{\partial p_{1e}}{\partial y}$ and $\frac{\partial p_{2e}}{\partial x}$ must have the same sign or be zero.

If this condition (5) is fulfilled,

$$P_z = \left[\frac{\partial P}{\partial x} \right]_{v,u} = \frac{1}{u_1} \frac{dp_1}{du_1} \left[x \frac{\partial u_1}{\partial x} + y \frac{\partial u_1}{\partial y} \right], \quad (6)$$

* Equation (3) may be obtained directly from the condition for a neutral equilibrium with respect to transformations at constant temperature and pressures, namely

$$F_{12} = F_1 + F_2.$$

For equilibrium to continue to exist for such transformations in the case of variations of the original systems,

$$dF_{12} = dF_1 + dF_2,$$

must hold for such variations, and since $dF = -SdT + vdp$, then at constant temperature these variations must meet the condition,

$$VdP = v_{1e}dp_{1e} + v_{2e}dp_{2e}.$$

However, this treatment does not bring out the fact that dp_{1e} and dp_{2e} are to be regarded as capable of independent variation. For example, if

$$F_1 = \frac{n_1 B}{n_1 B + n_2} F_{12},$$

and,

$$F_2 = \frac{n_2}{n_1 B + n_2} F_{12},$$

where B is a constant differing from unity,

then $F_{12} = F_1 + F_2$, but either n_1 and n_2 must be regarded as not independent or F_1 and F_2 and hence p_{1e} and p_{2e} must be regarded as not independent. Such a condition of equilibrium would be inconsistent with the requirement that each fluid be independently capable of being in equilibrium with the mixture and that this equilibrium not be disturbed by the addition or removal of the other fluid system. Such conditions for equilibrium may exist in the immediate neighborhood of an interface between fluids where concentration gradients exist; however, these considerations belong properly to the thermodynamics of surfaces, and will not be discussed further here.

Had we considered the equilibrium condition for transformations at constant temperature and volumes, namely,

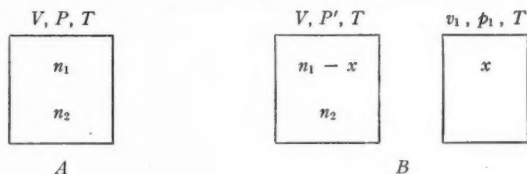
$$A_{12} = A_1 + A_2,$$

we would have arrived at an equation that is essentially similar in physical implications to Equation (3), namely,

$$PdV = p_{1e}dv_{1e} + p_{2e}dv_{2e} \quad (3a)$$

The physical significance of Equation (3) or (3a) may be illustrated as follows:

Let A and B be two systems at the same temperature which contain the same matter.



We may obtain B by a reversible process from A in any number of ways, e.g., by direct reversible isothermal expansion through a semipermeable membrane, or by decomposing A reversibly into pure n_1 and n_2 then dividing n_1 into $n_1 - x$ and x , then combining $n_1 - x$ and n_2 reversibly in any manner and eventually reversibly expand or compress each separate volume until the system B is obtained. ΔS for the process must be the same in the two processes. Otherwise we could operate a Carnot process in contradiction to the Second Law. Equations (3) and (3a) ensure that ΔS is independent of the path.

The Gibbs equilibrium conditions, $\mu'_1 = \mu_1$, etc., and the derivation of Equation (3) are, of course, very well known and are given in the standard texts. The derivations are repeated here in order to present a reasonably complete picture.

and,

$$P_y = \left[\frac{\partial P}{\partial y} \right]_{v,z} = \frac{1}{u_2} \frac{dp_2}{du_2} \left[x \frac{\partial u_2}{\partial x} + y \frac{\partial u_2}{\partial y} \right];$$

recalling the boundary conditions (Equation 2), $u_1 = x$ when $y = 0$, and $u_2 = y$

when $x = 0$, then $P_y = \frac{dp_2}{du_2} = \frac{dp_2}{dy}$ when $x = 0$, or

$$\int_0^y P_y dy = p_2(u) = p_2(y).$$

The pressure, P , of the mixture will be given by,

$$\begin{aligned} P &= \int_{x=0, y=y}^{x=x, y=y} P_x dx + \int_{x=0, y=0}^{x=0, y=y} P_y dy \\ &= \int_{x=0, y=y}^{x=x, y=y} \frac{1}{u_1} \frac{dp_1}{du_1} \left[x \frac{\partial u_1}{\partial x} + y \frac{\partial u_1}{\partial y} \right] dx + p_2(y). \end{aligned} \quad (7)$$

Thus if we know either u_1 or u_2 as a function of x and y and the equations of state of the two pure fluids, we can determine the equation of state of the mixture. Without a knowledge of u_1 or u_2 as a function of x and y we can tell nothing as regards the equation of state of the mixture.

In order to contrast Dalton's law and the Gibbs-Dalton law, which as we have seen are associated with these problems, we may express these two "laws" mathematically as follows:

Dalton's law,

$$P(x, y) = p_1(x) + p_2(y),$$

Gibbs-Dalton law,

$$P(x, y) = p_1(u_1) + p_2(u_2),$$

that is,

$$P = p_{1e} + p_{2e}.$$

Evidently when $u_1 = x$, and hence $u_2 = y$, the two laws become identical.

If we write,

$$\pi_1(x, y) = P(x, y) - p_1(x) - p_2(y),$$

then $\pi_1(x, y)$ is a measure of the departure from Dalton's law, and $\pi_1(x, y)$ is given by,

$$\pi_1(x, y) = \int_{x=0, y=y}^{x=x, y=y} \frac{1}{u_1} \frac{dp_1}{du_1} \left[x \frac{\partial u_1}{\partial x} + y \frac{\partial u_1}{\partial y} \right] dx - p_1(x).$$

While if we write,

$$\pi_2(x, y) = P(x, y) - p_1(u_1) - p_2(u_2),$$

$\pi_2(x, y)$ is a measure of the departure from the Gibbs-Dalton law and $\pi_2(x, y)$ is given by,

$$\pi_2(x, y) = \int_{x=0, y=y}^{x=x, y=y} \left[\frac{1}{u_1} \frac{dp_1}{du_1} \left[x \frac{\partial u_1}{\partial x} + y \frac{\partial u_1}{\partial y} \right] dx - p_1(u_1) - p_2(u_2) + p_2(y) \right] dx$$

When $u_1 = x$, and $u_2 = y$ for all values of the variables, $\pi_2(x, y) = 0$ and the Gibbs-Dalton law is obeyed, and when the Gibbs-Dalton law is obeyed Dalton's law necessarily holds. However, if $u_1 = x f_1(x, y)$, where $f_1(x, y)$ is homogeneous and of zero degree in x and y , and if each pure gas is ideal, i.e., $p_1 = K u_1$, $p_2 = K u_2$, then $\pi_1(x, y) = 0$ and Dalton's law is obeyed but not the Gibbs-Dalton law.

When,

$$P = p_{1e} + p_{2e}.$$

$$dP = dp_{1e} + dp_{2e},$$

but by (4),

$$dP = \frac{v_{1e}}{V} dp_{1e} + \frac{v_{2e}}{V} dp_{2e}$$

$$= \frac{x}{u_1} dp_{1e} + \frac{y}{u_2} dp_{2e},$$

and

$$\frac{v_{1e}}{V} = \frac{x}{u_1} = \frac{v_{2e}}{V} = \frac{y}{u_2} = 1,$$

i.e. $u_1 = x$, $u_2 = y$, and each fluid is as a vacuum to the other fluid regardless of its equation of state. And conversely when each fluid is as a vacuum to the other fluid (that is, $u_1 = x$, $u_2 = y$) then $dP = dp_{1e} + dp_{2e}$ and $P = p_{1e} + p_{2e}$. Evidently this is a highly restrictive condition and can only be regarded as a limiting possible case, rather than as a probable case for physical systems of the type we have been discussing.

It may be pointed out that even when $\frac{x}{u_1}$ and $\frac{y}{u_2}$ are not unity, by superimposing as an experimental condition,

$$dp_{1e} = \frac{(y - u_2)u_1}{u_2(u_1 - x)} dp_{2e},$$

we can find a path along which,

$$dP = dp_{1e} + dp_{2e},$$

and P becomes a function of p_{2e} only. But if $P = p_{1e} + p_{2e}$, in general, where p_{1e} and p_{2e} are independent variables, then,

$$\left(\frac{\partial P}{\partial p_{1e}} \right)_{p_{2e}} = 1 = \frac{x}{u_1},$$

and hence $x = u_1$, $y = u_2$. If, for example,

$$p_{1e} = \phi_1 P,$$

and,

$$p_{2e} = \phi_2 P,$$

then,

$$\phi_1 + \phi_2 = 1,$$

$$u_1 = x, \text{ and } u_2 = y,$$

or there is a functional relation between p_{1e} and p_{2e} (cf. footnote p. 996).

Discussion of Equations and Some Empirical Relations

The various equations presented above, although used to describe what we have called "membrane equilibrium" are in no way dependent on the existence or nonexistence of perfect semipermeable membranes. The treatment has been presented as though the three fluids were contained in three separate, impermeable containers and the equilibrium conditions deduced for variations of a type which would apply physically in the case of ideal membranes. Without reference to some physical process the whole discussion is, of course, practically devoid of meaning. That there are semipermeable membranes which in many cases are fairly close approximations to the ideal semipermeable membrane is unquestionable. When a pure crystalline compound is in equilibrium with a liquid or gaseous solution containing a common component, the interface between the crystalline material and the solution is an example of an almost perfect semipermeable membrane. In fact in many ways, the semipermeable membrane may be regarded as an idealized phase interface. Any mechanism by means of which a *selected* kind of material may be transferred from one volume to another by a reversible process may be regarded as a semipermeable membrane. Depending on the kind of "membrane", it may or may not be capable of supporting a pressure difference between the volumes concerned. The "membrane" must include all parts of systems where concentration or other gradients exist as between the two volumes concerned. The total mass of material in regions where such gradients exist must be small in comparison with the mass of material in the volumes under consideration in order that its variations may be neglected in comparison with the variations of the masses in the volumes concerned. In any case, the thermodynamic equilibrium must not, in any way, be dependent on the properties of the "semipermeable membrane" itself, except in so far as it is or is not capable of supporting a net pressure difference, and a net difference in thermodynamic potentials of selected materials or components. The fundamental condition that the thermodynamic potential of a pure material be the same as that of this material in the mixture or compound substance is the basic condition which virtually defines the semipermeable membrane. From this condition alone we can say practically nothing concerning the temperatures and pressures of the two volumes. However, to parallel real systems in contact with one another, the temperatures obviously must be the

same. While there seems little justification for making any assumptions about the pressures, we shall assume that the pressures are all positive and consider only cases where the pressure of the mixture is not less than the greatest of the equilibrium pressures of all pure systems with which it is in equilibrium.

Equation (5) imposes a considerable restriction on possible simultaneous forms of $f_1(x, y)$ and $f_2(x, y)$ in the equations $u_1 = xf_1(x, y)$, $u_2 = yf_2(x, y)$, although almost any form of either one alone which is consistent with the boundary conditions can be assumed without conflict with thermodynamic principles.

In many practical problems where deviations from the mole fraction law occur it is desirable to have some way of relating the pressures of two-component systems to the pressures of pure components in equilibrium therewith, or to obtain self-consistent relations between concentrations existing across semipermeable membranes in osmotic pressure experiments. In what follows we shall present a few explicit forms of $f_1(x, y)$ and $f_2(x, y)$ which are consistent with various equations of state and may prove useful for extrapolation purposes if not found of interest in themselves.

When $p_1(u_1) = Ku_1$, and $p_2(u_2) = Ku_2$, i.e., when both the pure fluids behave as ideal gases, Equation (5) reduces to,

$$\frac{\partial \log u_1}{\partial y} = \frac{\partial \log u_2}{\partial x},$$

and hence

$$\frac{\partial \log f_1}{\partial y} = \frac{\partial \log f_2}{\partial x}$$

expresses the necessary condition that a thermodynamic equilibrium exist.* If f_1 and f_2 meet this requirement and f_3 and f_4 also meet this requirement,

(i.e., $\frac{\partial \log f_3}{\partial y} = \frac{\partial \log f_4}{\partial x}$), then also,

$$\frac{\partial \log f_1 f_3}{\partial y} = \frac{\partial \log f_2 f_4}{\partial x}. \quad (8)$$

Hence, when individual gases are ideal or nearly so, any products or powers of pairs of explicit solutions of Equation (5) can also form a solution.† If these solutions are homogeneous functions of zero degree in x and y then the equation of state of the gas mixture must also be ideal. Thus,

$$u_1 = x \left(\frac{x+y}{x} \right)^D \cdot \prod_{i=1}^{i=n} \left(\frac{A_i x}{A_i x + B_i y} \right)^{A_i C} \cdot \prod_{j=1}^{j=m} e^{E_j (1-b_j) (y/x)^{b_j}}, \quad (9)$$

$$u_2 = y \left(\frac{x+y}{y} \right)^D \cdot \prod_{i=1}^{i=n} \left(\frac{B_i y}{A_i x + B_i y} \right)^{B_i C} \cdot \prod_{j=1}^{j=m} e^{E_j b_j (x/y)^{1-b_j}},$$

* Cf. the Gibbs-Duhem equation.

† In a purely mathematical sense.

where $A_i \geq 1$, $B_i \geq 1$, $C \geq 0$, $D \leq 1$, $E_i \leq 0$, $0 < b_i < 1$, constitute a general family of possible forms for u_1 and u_2 as functions of x and y , all of which lead to $P = K(x + y)$ and hence to Dalton's law. But of this infinite array of thermodynamically possible forms (it is not intended to imply that these are, by any means, the only such forms) only the special case $u_1 = x$, $u_2 = y$, is also consistent with the Gibbs-Dalton law and the Lewis partial pressure rule.*

The sum of the equilibrium pressures, P_{se} , will be given by,

$$P_{se} = p_{1e} + p_{2e} = p_1(u_1) + p_2(u_2) = Kx \cdot f_1 + Ky \cdot f_2,$$

which is, of course, not in general equal to P but may be greater or less than P .

When $D = 1$, and E and C are zero, in (9)

$$u_1 = x + y = u_2,$$

which corresponds to the case where the fluids are identical, and may be regarded as the limit of applicability of the boundary conditions and definitions. Here

$$P = K(x + y) = p_{1e} = p_{2e}.$$

Other special cases of interest are easily obtained. Thus (9) may be written,

$$u_1 = (x + y) \left(\frac{Bx}{Bx + y} \right)^{B^C}, \quad (10)$$

$$u_2 = (x + y) \left(\frac{By}{Bx + y} \right)^C.$$

When x and y are of comparable magnitude and $C = 1$ and $B \rightarrow \infty$ we get the limiting case $u_1 \rightarrow (x + y)e^{-y/x}$, $u_2 \rightarrow 0$ and $P \approx p_{1e} + p_{2e} \approx p_{1e}$, $p_{2e} \rightarrow 0$. Again, when x and y are of comparable magnitude and $B = 1$ and $C \rightarrow \infty$ we get the limiting case $u_1 \rightarrow 0$, $u_2 \rightarrow 0$ and $p_{1e} \rightarrow 0$, $p_{2e} \rightarrow 0$ and $P \gg p_{1e} + p_{2e}$.†

When $f_1(x, y)$, $f_2(x, y)$ are homogeneous functions but are not of zero degrees in x and y , in general Dalton's law will not hold. If the equilibrium conditions are such that,

$$u_1 = x \prod_{i=0}^{i=n} e^{C_i y (xy)^{a_i}}, \quad (11)$$

$$u_2 = y \prod_{i=0}^{i=n} e^{C_i x (xy)^{a_i}},$$

where $C_i \geq 0$ and $a_0 = 0$, $a_1 = 1$, $a_2 = 2 \dots \dots a_n = n$, and the pure fluids behave as ideal gases,

$$P = K \left[x + y + \sum_{i=0}^{i=n} C_i \frac{(2a_i + 1)}{a_i + 1} (xy)^{a_i + 1} \right].$$

* The Lewis partial pressure rule is assumed to be:

$$p_{1e} = \frac{x}{x + y} P, \text{ or } \mu_1' = f(T) + RT \log \frac{x}{x + y} P.$$

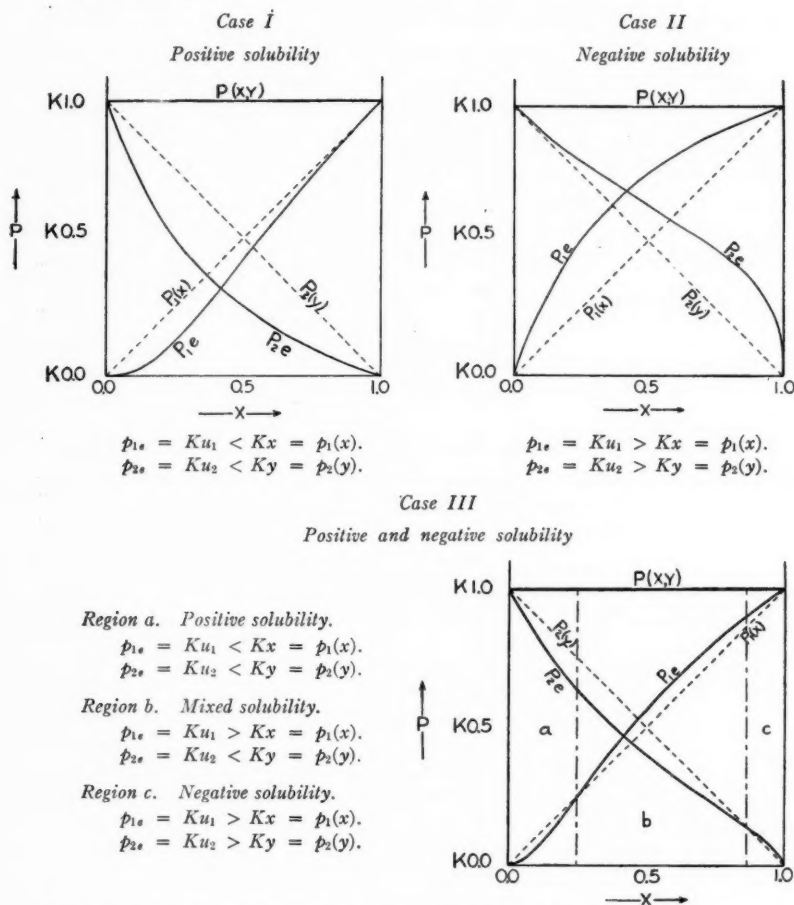
† Footnote on following page.

† The function f , although somewhat similar to an "activity coefficient" is, of course, by no means identical therewith. It might be regarded as a solubility coefficient at constant potential, the vacuum being taken as unity. When f_1 is greater than unity, x is less than u_1 , i.e., less than it would be if y were zero, and hence y exerts a "negative solvent" action compared to a vacuum. Similarly, when f_1 is less than unity y exerts a "positive solvent" action. Thus taking the simple empirical forms,

$$f_1 = \frac{x+y}{x} \left(\frac{3x}{3x+y} \right)^a,$$

$$f_2 = \frac{x+y}{y} \left(\frac{y}{3x+y} \right)^b,$$

We have three cases according to the values of a and b . Taking $a = 3$, $b = 1$, as Case I; $a = 1$, $b = 1/3$ as Case II; and $a = 2$, $b = 2/3$ as Case III; we get the following graphs, assuming $x + y = 1$ in each case.



It will be noted that $P = K(x + y)$, i.e., that Dalton's law holds in each of the three cases, but the equilibrium pressures are very different from the Lewis partial pressures $\frac{x}{x+y}P$ and $\frac{y}{x+y}P$. (In this case $\frac{x}{x+y}P$ and $\frac{y}{x+y}P$ are identical to $p_1(x)$ and $p_2(y)$, respectively.)

In the special case where $a_o = 0$ is the only term,

$$u_1 = xe^{Cy}, \quad u_2 = ye^{Cx}$$

and,

$$P = K(x + y + Cxy).$$

Functions of this type (11) yield symmetrical "interaction" terms. Of course there is no need for the "interaction" terms to be symmetrical. Unsymmetrical terms will be given by such expressions as,

$$u_1 = xe^{Cy^2}, \quad u_2 = ye^{2Cyx},$$

and,

$$P = K(x + y + 2Cxy^2).$$

When the pure gases are not ideal, u_1 and u_2 as functions of x and y which are consistent with the condition Equation (5) are much more difficult to find. Moreover, the relation expressed by (8) is no longer valid and we cannot readily obtain general solutions in closed parametric form. However, where observed relations between u_1 and x and y can be approximated by, say, $u_1 = xe^{Cy}$, and where

$$p_1 = Ku_1 + Au_1^2 + Bu_1^3 \dots,$$

$$p_2 = \text{any function of } u_2,$$

then,

$$P = K(1 + Cy)(x + A_1e^{Cy}x^2 + B_1e^{2Cy}x^3 \dots) + p_2(y).$$

Of course whatever the approximate form of the observed relations, $u_1 = xf_1(x, y)$ or $u_2 = yf_2(x, y)$, $P(x, y)$ as calculated from (7) must be consistent with the observed values for P .

That the Gibbs-Dalton law cannot be considered to apply generally to fluids which are not ideal gases may be illustrated by the following considerations.

Suppose at constant temperature two fluids have the same equations of state, namely,

$$p_1(u_1) = Ku_1 - Au_1^2 + Bu_1^3$$

$$p_2(u_2) = Ku_2 - Au_2^2 + Bu_2^3.$$

If these fluids are *different*, the Gibbs-Dalton law requires that for a mixture of them,

$$P = p_{1e} + p_{2e} = p_1(x) + p_2(y)$$

$$= K(x + y) - A(x^2 + y^2) + B(x^3 + y^3).$$

If, however, the fluids are identical we must have,

$$P = K(x + y) - A(x + y)^2 + B(x + y)^3$$

$$= p_1(x) + p_2(y) + \pi_1(x, y).$$

In this case $x + y$ represents the total concentration or $x + y = u$. The term $\pi_1(x, y)$ is equal to, $-2Axy + 3Bx^2y + 3Bxy^2$, and may be considerably larger than $p_1(x) + p_2(y)$.

If we hold the composition of the mixture of different fluids constant and vary the total concentration, $x + y$, we may speak of the P, u, T relations of the mixed fluid, u being equal to $x + y$. Evidently the fluid mixture has P, u, T , relations which are quite different from those of either pure fluid. We are thus led to a conclusion which is entirely contrary to experience, namely, that there should be a very marked difference between the P, u, T relations of a given pure fluid and the P, u, T relations of a solution consisting of roughly equal parts of the given fluid and of a second fluid, however small the difference between the two fluids might become.

Evidently the function $f_1(x, y)$ in $u_1 = xf_1(x, y)$ must supply the interaction terms which cannot be lacking in fluid mixtures when the pure fluids are very similar and have nonlinear equations of state.

III. Physical Implications

Equilibrium Pressures; Illustrations

Referring to Fig. 1, when the distributions of the two fluids across the membranes are completely independent so that each fluid acts as a vacuum to each other i.e., when $P = p_{1e} + p_{2e}$, we may decrease the volume v_{1e} , thereby increasing the pressure p_{1e} and the values of u_1 and x by any finite quantity, without altering u_2, y, p_{2e} or v_{2e} . When, however, u_1 is not independent of y , then any finite change in p_{1e} will cause a change in p_{2e} if the volumes V and v_{2e} are kept constant. Thus the system behaves physically, as though the pressure p_{1e} of the pure gas in v_{1e} were partially transferable to the pure gas cell containing the second species. In such cases the pressure in V will be given by $P \geq p_{1e} + p_{2e}$. To illustrate what is involved when the fluids are not independent with respect to the pressures they transmit across the interfaces or semipermeable membranes, and referring again to Fig. 1, let us suppose that the two fluids are in fact independent, so that, $P = p_{1e} + p_{2e}$; and we introduce a third independent fluid into the system. If both membranes are permeable to this third fluid and we represent its equilibrium pressure by p_x , then the new pressure p'_{1e} in v_{1e} will be $p'_{1e} = p_{1e} + p_x$, and similarly $p'_{2e} = p_{2e} + p_x$ and $P' = P + p_x$, where p'_{2e}, P' are the corresponding new pressures in v_{2e} and V , respectively. Now we find that $p'_{1e} + p'_{2e}$ is greater than P' since the added pressure p_x is counted twice in the sum $p'_{1e} + p'_{2e}$. If we had added the third gas only to V , i.e., to the compartment containing the mixture and had assumed the third fluid to be incapable of passing either membrane we would find $p'_{1e} + p'_{2e}$ less than P' . These relations are somewhat analogous to the interaction of fluids which are not independent; if the second component in the mixture can transmit a "force" across the membrane on the pure first component, then the first component in the mixture must exert a force on the second pure component and this will introduce a correction on the additivity

of the equilibrium pressures similar to the case where the ideal fluid passes both diaphragms. On the other hand, the interaction of the two fluids may give rise to other substances, molecular groups or "forces" which cannot pass either diaphragm. In a particular composition these two opposing tendencies might just cancel, and the system at this particular point behave as though independent. As far as real systems are concerned both of these cases should occur quite generally. When the fluids are dilute but react chemically we should expect $P > p_{1e} + p_{2e}$ and where the fluids are fairly concentrated and do not react appreciably, $P < p_{1e} + p_{2e}$. When the fluids are completely insoluble or identical, $P = p_{1e} = p_{2e}$. As an example of the first case, where $P > p_{1e} + p_{2e}$, we might take the case of H_2 and I_2 in equilibrium through semipermeable membranes with HI , H_2 , and I_2 , while as an example of the second case we might suppose the first fluid to be liquid water and the second fluid to be liquid mercury, the diaphragms being permeable to water and mercury respectively. Assuming these liquids completely insoluble in one another, and within the mixed compartment—any water in contact with the water-permeable membrane—any mercury in contact with the mercury-permeable membrane—then the only condition consistent with equilibrium is, $p_{1e} = P = p_{2e}$. In this case p_{1e} must equal p_{2e} and they are completely dependent in the sense that it is not possible to vary one by a finite quantity without varying the other by exactly the same amount as long as the pressures lie within the range of existence of the mutually insoluble liquid phases. In this sense, when the two fluids become identical, again p_{1e} and p_{2e} are completely dependent and $p_{1e} = P = p_{2e}$. But in this case there is no limit to the range of complete dependence, being true at all pressures.

The net pressure across a semipermeable membrane will be $P - p_{1e}$, where the membrane is permeable to the first fluid and p_{1e} is the equilibrium pressure of the first fluid.* In the case of a mixed system in equilibrium with two fluids, $P - p_{1e}$ may be greater or less than p_{2e} according as P is greater or less than $p_{1e} + p_{2e}$. For example, if diaphragm (1) is impermeable to a

* Of course, not, in general, p_{2e} . The common assumption that the net pressure exerted on the diaphragm is p_{2e} can lead to all sorts of absurdities. For example, referring to Fig. 1, assuming the net pressure sustained by diaphragm (1) to be p_{2e} and that sustained by diaphragm (2) to be p_{1e} , then if we move the diaphragms toward one another, thereby tending to separate the fluids in the mixed compartment, we must do work on the system. Since nothing has been said concerning the natures of the diaphragms and fluids, we may suppose the two fluids to be entirely different insoluble liquids, or two liquid phases of different compositions (e.g., water-ether, ether-water solutions) both in equilibrium with the same vapor, or we might suppose the first fluid to be a liquid and the second to be its vapor. We might thus be led to the absurd conclusion that there was a large "entropy of mixing" associated with the removal of a partition separating two fluids already in a state of complete thermodynamic equilibrium. Of course in all of the above cases the net pressures on the fictitious diaphragms are $P - p_{1e} = 0 = P - p_{2e}$. The mere movement of the diaphragms even if accompanied by a definite experimentally identifiable change in distribution of matter does not necessarily involve the performance of work when carried out reversibly.

The validity of such arguments is not so much a matter of the experimental possibility or impossibility of separating mixtures by means of such membranes, but is rather a question as to whether such "separations" conform analytically to the idealizations implied in the mathematical operations, $(\frac{\partial A_{12}}{\partial n_1})_{T,V,n_2}$, $(\frac{\partial A_1}{\partial n_1})_{T,V,n_2}$, etc. These operations include the case where n_1' and n_2' are arbitrary divisions of a single variable n , i.e., the case of identical fluids.

liquid but permeable to its vapor, it is possible for a net pressure to exist across the diaphragm (not, perhaps, strictly a condition of absolute physical stability). Let the net pressure be p_x , then $p_x = P - p'_{1e}$, where p'_{1e} is the vapor pressure of the liquid under these conditions. Now p_x might be a pressure due to an insoluble inert gas on the liquid side of the diaphragm (1). If the gas acts as a vacuum to the vapor of the liquid and p_{2e} is the equilibrium pressure of this inert gas, then $p_x = p_{2e}$, since $P = p_x + p'_{1e} = p_{2e} + p'_{1e}$. But if the vapor and inert gas are not mutually independent $p_x \geq p_{2e}$ and the net pressure across diaphragm (2) is $P - p_{2e} = p'_{1e} + p_x - p_{2e} \geq p'_{1e}$.

If the "membrane" cannot support a net pressure toward the mixture, as might be the case when the "membrane's" only function is to prevent passage of part of the mixture toward the pure fluid, then P cannot be less than the greater of p_{1e} and p_{2e} and, hence, the smallest value of $P/(p_{1e} + p_{2e})$ is one-half, while the greatest value is unlimited. However, using the expression "membrane equilibrium" in its most general sense P might be less than either one of p_{1e} and p_{2e} . In any case, there is certainly no thermodynamic justification, whatever, for the assumption that $p_{1e} = P - p_{2e}$.

Thermodynamic Quantities

As we have pointed out in the first part of this paper the expression "the entropy of mixing" is usually used to denote the entropy change associated with the combining of two separate volumes of fluids into a single fluid mixture whose volume is equal to the sum of the original volumes. Let us, rather, consider the changes, if any, in the thermodynamic quantities associated with the converting isothermally of n_1 moles of the first kind of fluid and n_2 moles of the second kind when each alone is contained within a volume V , into a mixture containing the same matter in an equal volume V .

In order to combine the gases reversibly we must first expand or compress each gas separately until each separate gas is in such a state as to be in thermodynamic equilibrium with the mixture to be formed. Then the two gases are to be combined reversibly, the two volumes disappearing isothermally under their respective equilibrium pressures p_{1e} , p_{2e} while a single volume of mixture under its equilibrium pressure P makes its appearance.

The total heat received will consist of two parts. First there will be the heat received in bringing each fluid to its equilibrium state, namely,

$$T\Delta S_1 = \int_V^{v_1} dE_1 + \int_V^{v_1} p_1 dv_1 + \int_V^{v_2} dE_2 + \int_V^{v_2} p_2 dv_2.$$

Second, there will be the heat absorbed in the equilibrium conversion, namely,

$$T\Delta S_e = E_{12} - (E_{1e} + E_{2e}) + PV - (p_{1e}v_{1e} + p_{2e}v_{2e}).$$

While the observed value of the total heat received in the two processes divided by the temperature would measure the total entropy change, $S_{12} - (S_1 + S_2)$, the equations of state in terms of P , u , and T will not enable us to

calculate the entropy change even if we know the equilibrium conditions, since we must also know the absolute value of the energies* as functions of the volumes for the pure fluids as well as for the mixture.

When $u_1 = x$, $u_2 = y$, represent the equilibrium conditions then, as shown by Gibbs, all of the thermodynamic quantities are additive and $T\Delta S_1 = T\Delta S_e = 0$, regardless of the equations of state of the individual fluids.

When each pure gas, as well as the mixture, is ideal in the sense that $pV = nRT$ and $E = 3/2 nRT$, then $T\Delta S_e = 0$, regardless of whether $u_1 = x$, $u_2 = y$, or not. However, $T\Delta S_1$ will not be zero when u_1 and u_2 are not equal to x and y respectively. When both gases are ideal and the equilibrium conditions require equal concentrations on either side of the semipermeable membranes then $\Delta S = 0$ for the entire process of combining equal volumes into a single equal volume. Each gas is then behaving as a vacuum to the other gas. We should contrast this limiting case of completely independent ideal gas mixtures with the limiting case of completely dependent ideal gas mixtures. In the latter case $p_{1e} = P = p_{2e}$ and the only condition consistent with this is $u_1 = x + y = u_2$, and since this implies that $n_1 = n_2$, ΔS becomes equal to $-R \log 2$ per mole.

The entropy change which accompanies the removal of a partition between two fluids, having the same pressure and temperature, is to be obtained in the same way except that in place of the lower limit V in the first integration we use V_1 and V_2 respectively, V_1 and V_2 corresponding to the initial fluid volumes and $V_1 + V_2 = V$. Where E and (PV) are constant, as would be the case for ideal gases whose mixtures obey Dalton's law and where $E_{12} = E_{1e} + E_{2e}$, the entropy of mixing will be given by,

$$\Delta S = RV \left[x \log \frac{x+y}{u_1} + y \log \frac{x+y}{u_2} \right],$$

where u_1 and u_2 must be homogeneous functions of first degree in x and y . Putting in the values of u_1 and u_2 from, say, (10) we get,

$$\Delta S = RV \left[x \log \left(\frac{Bx+y}{Bx} \right)^{Bc} + y \log \left(\frac{Bx+y}{y} \right)^c \right].$$

When the equilibrium pressures are completely "dependent", as would be the case were the fluids identical, $C = 0$ and the entropy of mixing vanishes, as it should. When the equilibrium pressures are completely independent, then $B = C = 1$, and we get the classical entropy of mixing. When x and y are of comparable magnitude and either B or C are large we get large values for the entropy of mixing. Thus comparing the entropy of mixing associated with the removal of the partition, in the limiting cases of (a) complete "dependence" of pressures, (b) complete independence, and (c) the limiting

* In other words we must know ΔE for the process. When dealing with mechanical model gases we usually assume absolute energies although such absolute energies have little or no meaning thermodynamically (cf. footnote, p. 1009).

cases where B or C approach infinity, we have (a) no entropy change, (b) an entropy change corresponding to $RV \left[x \log \frac{x+y}{x} + y \log \frac{x+y}{y} \right]$, (c) an infinite entropy change.

Real fluids may be expected to show an entropy of mixing varying between these limits. Of the three limiting cases, evidently, the first is the suitable choice of limiting condition from which real systems may be expected to deviate as the fluids become physically distinguishable from one another, since this condition must hold for identical fluids and may be closely approached in many cases when fluids are either very similar or insoluble. In any case, it is evident that by introducing the equilibrium condition, which is absolutely essential to any calculation of the entropy of mixing, we avoid a good many of the difficulties of the Gibbs Paradox.

It is to be emphasized that the relations we have put forward in Part II of this paper apply without any reservations concerning the energies of the gases involved, and apply to cases where ΔE is zero for the mixing process as well as to cases where ΔE is not zero. Consequently when Dalton's law is obeyed and the gases are all ideal, ΔH for the mixing process may have almost any value. The relations of Equation (9) are thus perfectly applicable to cases where ΔH is zero as well as to cases where ΔH is not zero.

When two dilute diatomic gases A_2 and B_2 , each of which obeys Boyle's law closely, react to form the gas AB , which also obeys Boyle's law, we might have an example of a case when p_{1e} and p_{2e} were small compared with P , while Dalton's law was obeyed. Depending on the relative values of the bond energies $A-A$, $B-B$, and $A-B$, such reactions may or may not be associated with a large ΔE and hence a large ΔH . While large negative values of ΔF are usually associated with large negative values of ΔH , $(-\Delta F)$ may be quite large when ΔH is practically zero. In such cases there is a large positive entropy change accompanying the reaction. Thus ΔE and ΔH might be zero for such a reaction as $A_2 + B_2 = 2AB$, while $p_{1e} \ll P$ and $p_{2e} \ll P$, and there will be a large entropy of "mixing" or "reaction". It is also perfectly permissible thermodynamically that $p_{1e} \approx P$ and $p_{2e} \ll P$, when ΔE and ΔH are zero, but these relations are difficult to interpret in terms of any sort of ordinary chemical reaction. Such relations seem to imply that it is possible for the relative capacities for thermal energy of constituents to change on mixing. Cases where $p_{1e} > p_1(x)$, $p_{2e} < p_2(y)$ should be observable in the case of ideal gas mixtures but can hardly be due to any ordinary chemical reaction.

When surface forces are not excluded the situation may be quite different. Thus, if in a real gas mixture, the value of $(-\Delta F)$ for the reaction of each gas alone with the wall of the container is large in comparison with $(-\Delta F)$ for the reaction of the gases themselves, and the rate of exchange of energy of each gas with the wall is large in comparison with the rate of exchange of energy between the gases themselves, then each gas can be more or less independently in a state of equilibrium with the wall. In the limiting case, each

gas can act as a vacuum to the other gas. Even identical gases may show an entropy of mixing, under such extreme circumstances, owing to a change in surface volume ratio. Such a situation might prevail in a very dilute gas mixture contained within a comparatively small volume, where the mean "free path" of the gas molecules was large in comparison with the dimensions of the container. Under these circumstances the whole gas is dominated by the wall reaction equilibrium and the free energies, energies, entropies of the fluid are by no means small compared to surface free energies, etc. For more concentrated systems such a condition might prevail in the immediate neighborhood of the surface.

When p_{1e} and p_{2e} are independent, in the sense which we have been using, the other thermodynamic properties are also independent and in such cases we may speak of the energy, entropy, etc., of a constituent of a mixture. Although even in this case the total work which can be done in an adiabatic expansion through a semipermeable membrane is equal to the whole of the thermal energy of the mixture, and hence we might regard the energies of each component as being the same, assuming all of the energy to be thermal energy. When, however, p_{1e} and p_{2e} are not independent the other thermodynamic properties are not independent and the assigning of energies, entropies, etc., to components within a mixture becomes highly arbitrary. Such relations as those of Equation (10) indicate that even in the case of an ideal gas mixture where $PV = (n_1 + n_2)RT$ and $E = (n_1 + n_2)3/2RT$ and where $n_1 = n_2$ the thermodynamic potentials of each component may be very different.* When such a difference exists it seems inescapable that if there is to be any meaning to the notion of the free energy per mole of a constituent within a mixture, then these free energies must differ. While we might similarly suppose, in such cases, that the energies per mole must also differ, it must be borne in mind that we might equally well suppose that the mean energies were the same but that the entropies differed. Evidently such distinctions have an arbitrary character from a thermodynamic point of view. If we regard all of the energy as kinetic energy of translation it is evident that there is no purely thermodynamic requirement that mean kinetic energies of different kinds of gas molecules in gas mixtures shall be the same. In fact the limiting case of Equation (10) where B approaches infinity, and $C = 1$, would indicate that thermodynamically it would be perfectly possible that one kind of gas have practically all of the kinetic energy, while the second kind have practically none, even when the molecules of each were equally numerous. The fact that such a partition of kinetic energy makes the entropy of the mixture a maximum and thus in a certain sense "most probable" is no justification for ignoring the physical constraints necessarily present in real gas mixtures and thus concluding that real gas mixtures which approximate the ideal gas laws will behave in any such manner. It does indicate, however, that such limiting cases are thermodynamically possible and that, depending on the detailed

* Strictly, comparisons of thermodynamic properties of fluids are meaningless unless the fluids are convertible into one another reversibly. Here it is assumed that the pure gases have the same potentials when having the same T and P .

physical nature of the gases, it is quite possible thermodynamically for real gases which obey the ideal gas laws and Dalton's law, to show an entropy of mixing varying from zero to large values, even when $\Delta H \approx 0$ for the process.

If we wish a detailed mechanical "explanation" or model of such thermodynamic relations, obviously we must seek such a model outside of the field of thermodynamics and consequently such discussions do not belong here. However, it may not be out of place to mention briefly one or two points that have some slight bearing on such problems.

It will be immediately evident that if we suppose that all of the energy of every ideal gas is wholly kinetic energy of translation and that at a given temperature every such pure gas has a Maxwellian distribution of velocities about a modulus proportional to the temperature, and every constituent of a gas mixture has the same velocity distribution, the constituent gas and pure gas are identical and hence each such gas is as a vacuum to every other such gas. If, however, we only require that the energy distribution of the whole gas mixture be Maxwellian and permit constituent gases to have non-Maxwellian velocity distributions, relations such as those of Equation (10) where $B > 1$, $C = 1$, would not be excluded. In this case the forms of the velocity distributions of the constituent gases could hardly be consistent with statistical independence of velocity components. In any case it is evident that there is no purely thermodynamic requirement that each constituent in a gas mixture have a Maxwellian distribution of velocities when the whole gas has its energy distributed canonically or is Maxwellian with respect to its kinetic energy.

Metastable Equilibrium and Boundary Conditions

When we write the condition for equilibria, as regards distribution of material across a semipermeable membrane in the form,

$$u_1 = x \cdot f(x, y) \quad \text{or} \quad u_1 = (x + y)f'(x, y),$$

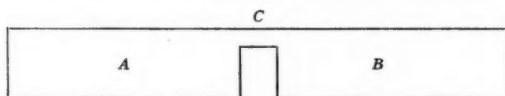
and suppose that $u_1 = x$ when $y = 0$ is a necessary condition which we can impose as a boundary condition on $f(x, y)$ we are assuming that we are not in a range of values where u_1 itself is indeterminate. Writing the equation of state in the form,

$$P = P(u),$$

at constant temperature, there may be not more than three values of u consistent with a given pressure and temperature. Since there may be any quantity of each phase the value of u within a container containing more than one phase is indeterminate as far as equilibrium conditions are concerned. When the temperature is such that there may be two values of u consistent with a given pressure P_s , as we increase the value of u , P will increase until the pressure P_s is reached after which (if the second phase is present in small but finite quantities) the value of u may be increased by a large factor—a thousandfold or more in many cases—and P_s will remain constant. (In the complete absence of the second phase the pressure will normally continue

to increase smoothly and undergo no distinctive change in the neighborhood of the point P_* .) Thus u may be not completely defined in terms of P and T in such regions.

As far as membrane equilibrium is concerned, (i.e., equilibrium with respect to the distribution of mass between volumes) when T and P have such values that two or more states may coexist, the distribution of matter across a permeable membrane or across an imaginary plane becomes essentially indeterminate. In this region infinitesimal forces or purely passive resistances may permanently stabilize one state as compared with another. The indeterminate character of such systems may be simply illustrated as follows:



Let A and B represent two large shallow closed vessels connected by means of a narrow passage C , the whole being immersed in a thermostat. If initially we place solid tungsten, copper, or liquid mercury in B and assume that A contains the corresponding vapor at the equilibrium pressure, a comparatively permanent state will exist where the concentration of material in B is vastly greater than in A , even when very considerable adventitious variations in temperature and external fields occur. The distribution of mass will be the more nearly perfectly stationary, the larger the passive resistances and the smaller the variations in the external influences. If, however, we had placed liquid water in B and water vapor, at the equilibrium pressure, in A , the permanency of the unequal mass distribution will be largely determined by external circumstances, the distribution tending to equalize rapidly if there are fairly large temperature variations as between A and B where such variations produce no net tendency toward an unequal distribution. A comparatively permanent unequal distribution would result from a number of small influences, such as a trace of soluble material in one compartment, a gravitational field in the direction of $A \rightarrow B$, a minute net temperature difference, etc. If completely shielded from all outside influences, it would require a great length of time to achieve complete equality of density in the two vessels. While these systems may, perhaps, be regarded as not "stable" in a statistical sense but only "metastable" they are essentially cases of indeterminate equilibria and exhibit a number of properties characteristic of these regions. In such regions the observed distribution of material will be determined by the magnitude of the passive resistances to change in comparison with the magnitudes of the adventitious variations in external forces. When the passive resistances are small the behavior will be characteristic of the external conditions, (e.g., the nature of the variations of the "temperature" of the thermostat, the gravitational effects, etc.) rather than characteristic of the material itself. These conditions would prevail at the "critical temperature". Here the uncertainty in density distribution would not be as great as would be the

case at temperatures below the critical temperature, but the sensitivity of the system to external agencies would be greater, the passive resistances being, in general, less. In none of these cases is the distribution of matter a characteristic of the thermodynamic equilibrium. Thermodynamically the dense and attenuated phases, while not identical, are identical with respect to any tendency toward change (their values of E and S per unit mass are not the same, but their values of $E - TS$ per unit mass are the same) as are all mixtures thereof, which do not introduce appreciable surface free energies. Thermodynamically the concentrations are not defined in such regions. Any processes which tend to change the distribution of matter as between the two vessels when in a state of thermodynamic equilibrium are not finite thermodynamic processes since no finite ΔS , ΔE , ΔF , etc., can be assigned to them.

Returning to the membrane equilibrium equation,

$$u_1 = x \cdot f_1(x, y),$$

not only will this relation not be defined when there can be more than one value for u consistent with a given value of p_{1e} and T , but the mixture containing x and y may also be capable of other values. A given mixture (x, y) may have such values of P and T that a second system (x', y'), where x' and y' are much greater than x and y , respectively, is capable of coexistence with the less dense mixture (x, y) and hence with u_1 .

We can apply Equation (7) if we know the complete equations of state $p_1(u_1)$, $p_2(u_2)$, and u_1 as a function of x and y for all values for the variables. But generally we do not know these equations completely. However, if we specify that u_1 , u_2 , x , and y always refer to the smallest densities consistent with given values of p_{1e} , p_{2e} , and T , we can retain the boundary conditions, $u_1 = x$ when $y = 0$, and $u_1 = 0$ when $x = 0$, as well as using approximate equations of state and an approximate relation $u_1 = x \cdot f_1(x, y)$, all of which shall be of reasonable validity for the small values of x and y and so obtain P as a function of x and y which is also of reasonable validity.

While these boundary conditions have been discussed with relation to semipermeable membranes, they are not primarily dependent on such membranes but are related to what is inferred in the whole structure of thermodynamics, namely, that an equation of state is applicable to bodies of matter and that an equation of continuity is applicable to thermodynamic processes. The first assumption regarding the equation of state may be put in many forms, one of which is that if two bodies consist of the same mass and of the same kind of matter, and each body is under the identical external forces (temperature, pressure, e.m.f., etc.), and if each body occupies the greatest volume consistent with its being in a state of reversible equilibrium with these forces, the volumes will be the same, the energies, entropies, etc., will be the same. Similarly, if the volumes are the same, the external conditions the same, and each contains the same kind of matter and the smallest mass consistent with its being in a condition of reversible equilibrium with its environment, the masses, etc., will be the same. Again, if each have the greatest entropy consistent with its

volume, mass, etc., the energies, etc., will be the same. The second underlying assumption is that if one of the bodies contain an infinitesimal mass of another kind of material which when pure has a finite density, energy, etc., under the given external conditions, the greatest volumes of the two masses, one pure and one impure, consistent with their being in a state of reversible equilibrium with the external conditions, cannot differ by more than an infinitesimal, the energies, entropies, etc., cannot vary by more than an infinitesimal. The differences must approach zero continuously as the quantity of the impurity approaches zero. Thus to each body of matter an equation of state of the form

$$\phi(T, P, X, V, M, \mu_1, \mu_2, \dots) = 0$$

is applicable, where T is the temperature, P the pressure, X other external forces, V the volume, M the mass and μ_1, μ_2 , etc., are the thermodynamic potentials of each kind of matter comprising the whole mass M .

IV. Concluding Remarks

It is obvious that ordinary liquids do not obey the Gibbs-Dalton law even approximately. It is equally obvious that gases which obey Boyle's law very closely but which react chemically do not obey this law. Gillespie (2), McHaffie (3), and others have shown that the Gibbs-Dalton law does not hold for such gas mixtures as $H_2 + NH_3$, $N_2 - NH_3$, $H_2O - \text{air}$, etc., the concentration of H_2O or NH_3 being as much as 50% higher and in some cases appreciably lower than required by the Gibbs-Dalton law. The classical experiments of Dalton, Reguault, and Magnus are to be regarded as approximate verifications of Dalton's law, not the Gibbs-Dalton law. As far as we are aware, very few experiments have been carried out in order to ascertain whether the Gibbs-Dalton law holds in regions where the gases are reasonably close approximations to ideal gases which do not react chemically in the ordinary sense. These regions are, by far, the most interesting regions in which to test the validity of the Gibbs-Dalton law. If appreciable deviations were found in cases of nearly ideal inert gas mixtures it would permit the various contributing factors to be correlated much more readily than would be the case were the gases not nearly ideal, and thus might lead to the discovery of some hitherto unknown general relations.

While we have perhaps overemphasized the fact that the Gibbs-Dalton law is not generally valid and, without considerable qualifications has neither a theoretical nor an experimental basis, Gillespie seems to have interpreted Gibbs' statements in this connection as implying that the law is generally applicable to fluid mixtures. It is our opinion that Gibbs meant this law as generally valid only in the case of mixtures of perfect inert gases. When so restricted the law is in conformity with classical kinetic theory, i.e., to what is usually assumed concerning the mixing of Maxwellian gases, as well as being in conformity with the usual application of modern statistics to such problems. However, it must be pointed out that the Maxwellian gas, the Einstein-Bose gas, the Fermi-Dirac gas, etc., are all model thermodynamic

fluids, and, in so far as the theoretical treatment of mixtures of these model fluids which obey Dalton's law exactly, requires the Gibbs-Dalton law to be obeyed, the theoretical treatment goes beyond thermodynamic requirements, and hence beyond the thermodynamics the models are intended to illustrate. While such theoretical treatment may often be valid, it is our opinion that it is too restrictive and should not be regarded as generally valid without a great deal more experimental evidence. It is thought that many cases can be found where real gas mixtures obey Dalton's law quite closely and where the energies are additive but the entropies not additive. Moreover, it is thought that among those cases where the thermodynamic potentials of the constituents are changed as a result of the mixing, there will be cases where the thermodynamic potential of one constituent is increased while that of the other constituent is decreased, as well as the more generally recognized case of "chemical" reaction, where both thermodynamic potentials are decreased. In other words, when Dalton's law is obeyed it is quite possible thermodynamically that the equilibrium pressure of a given constituent gas may be greater than the pressure which the same gas would exert if it occupied the space alone. Accordingly such cases should be observable. However, there may be reasons apart from thermodynamics why such cases cannot occur.

If it is, indeed, a general experimental fact that deviations from the Gibbs-Dalton law are always negligible when deviations from Dalton's law are negligible, and where there is no evidence of chemical reaction, in the ordinary sense, then Dalton was essentially right in stating that different gases act as a vacuum to one another. If, however, this is not generally true, as what little experimental evidence there is seems to indicate, then the paradox concerning the entropy of mixing is largely an illusion, and theoretical treatments of statistical model mixtures which assume that the statistics of constituents in mixtures are mutually independent are not necessarily valid even in cases where each pure constituent gas is ideal as well as the gas mixture.

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